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(54) Title: PRODRUGS OF THROMBIN INHIBITORS

(57) Abstract

There is provided compounds of formula (I): R^1O(O)C-CH$_2$-(R)Cgl-Aze-Pab-R^2, wherein R^1 and R^2 have meanings given in the description, which are useful as prodrugs of inhibitors of trypsin-like proteases, such as thrombin, and in particular in the treatment of conditions where inhibition of thrombin is required (e.g. thrombosis) or as anticoagulants.

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Prodrugs of Thrombin Inhibitors

Field of the Invention

This invention relates to pharmaceutically useful prodrugs of pharmaceutically active compounds, which active compounds are, in particular, competitive inhibitors of trypsin-like serine proteases, especially thrombin, the use of the prodrugs as medicaments, pharmaceutical compositions containing them and synthetic routes to their production.

Background

Blood coagulation is the key process involved in both haemostasis (ie the prevention of blood loss from a damaged vessel) and thrombosis (ie the formation of a blood clot in a blood vessel, sometimes leading to vessel obstruction).

Coagulation is the result of a complex series of enzymatic reactions. One of the ultimate steps in this series of reactions is the conversion of the proenzyme prothrombin to the active enzyme thrombin.

Thrombin is known to play a central role in coagulation. It activates platelets, leading to platelet aggregation, converts fibrinogen into fibrin monomers, which polymerise spontaneously into fibrin polymers, and activates factor XIII, which in turn crosslinks the polymers to form insoluble fibrin. Furthermore, thrombin activates factor V and factor VIII leading to a "positive feedback" generation of thrombin from prothrombin.

By inhibiting the aggregation of platelets and the formation and crosslinking of fibrin, effective inhibitors of thrombin would therefore be expected to exhibit antithrombotic activity. In addition, antithrombotic activity would be expected to be enhanced by effective inhibition of the positive feedback mechanism.

Prior Art

The development of low molecular weight inhibitors of thrombin has been described by Claesson in Blood Coagul. Fibrin. (1994) 5, 411.

Blombäck *et al* (in J. Clin. Lab. Invest. 24, suppl. 107, 59, (1969)) reported thrombin inhibitors based on the amino acid sequence situated around the cleavage site for the fibrinogen Aα chain. Of the amino acid sequences discussed, these authors suggested the tripeptide sequence Phe-Val-Arg would be the most effective inhibitor.

Low molecular weight peptide-based thrombin inhibitors have subsequently been disclosed in, for example, US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 94/29336, WO 93/18060 and WO 95/01168; and European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642 and 601 459.

More recently, thrombin inhibitors based on peptide derivatives have been disclosed in European Patent Application 0 669 317 and International Patent Applications WO 95/23609, WO 95/35309, WO 96/25426 and WO 94/29336.

In particular, the latter application discloses the peptide derivatives $R^aOOC-CH_2-(R)Cgl-Aze-Pab-H$, wherein R^a represents H, benzyl or C_{1-6}

alkyl.

Although these active compounds are known to exhibit significant antithrombin activity, it would be beneficial to improve their pharmacokinetic properties both after oral and parenteral administration. Examples of pharmacokinetic properties which it is desirable to improve include:

(a) providing an improved absorption from the gastro-intestinal tract, with a view to reducing intra- and/or inter-individual variability in relation to the bioavailability of the active compounds;

(b) flattening the plasma concentration time profile (ie reducing the peak/trough ratio in the plasma concentration over the dosing interval), with a view to reducing the risk of falling outside the therapeutic interval and the side effects caused by a concentration peak which is too high (eg bleeding), and those caused by one which is too low (eg thrombus formation); and

(c) increasing the duration of action of the active compounds.

Moreover, oral and parenteral administration of active thrombin inhibitors may lead to undesirable local bleeding (eg in the intestinal lumen or subcutaneously) as a result of a high local concentration,

Finally, orally administered active thrombin inhibitors which also inhibit trypsin and other serine proteases in the gastrointestinal tract may exhibit additional side effects, including indigestion (eg if trypsin is inhibited in the intestinal lumen).

Although certain N-benzyloxycarbonyl derivatives of the aforementioned active compounds are also disclosed as thrombin inhibitors in International Patent Application WO 94/29336, that these derivatives may be useful as prodrugs is not mentioned. In fact, WO 94/29336 makes no mention of suitable prodrugs of the active compounds.

We have found that the above problems may be solved by administering compounds according to the present invention which, whilst inactive *per se*, upon oral and/or parenteral administration are metabolised in the body to form active thrombin inhibitors, including those mentioned above.

Disclosure of the Invention

According to the invention there is provided a compound of formula I,

$$R^1O(O)C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}R^2 \qquad \text{I}$$

wherein

R^1 represents $-R^3$ or $-A^1C(O)N(R^4)R^5$ or $-A^1C(O)OR^4$;

A^1 represents C_{1-5} alkylene;

R^2 (which replaces one of the hydrogen atoms in the amidino unit of Pab-H) represents OH, $OC(O)R^6$, $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$;

R^3 represents H, C_{1-10} alkyl, or C_{1-3} alkylphenyl (which latter group is optionally substituted by C_{1-6} alkyl, C_{1-6} alkoxy, nitro or halogen);

R^4 and R^5 independently represent H, C_{1-6} alkyl, phenyl, 2-naphthyl or, when R^1 represents $-A^1C(O)N(R^4)R^5$, together with the nitrogen atom to which they are attached represent pyrrolidinyl or piperidinyl;

R^6 represents C_{1-17} alkyl, phenyl or 2-naphthyl (all of which are optionally substituted by C_{1-6} alkyl or halogen);

R^7 represents 2-naphthyl, phenyl, C_{1-3} alkylphenyl (which latter three groups are optionally substituted by C_{1-6} alkyl, C_{1-6} alkoxy, nitro or halogen), or C_{1-12} alkyl (which latter group is optionally substituted by C_{1-6}

alkoxy, C_{1-6} acyloxy or halogen);

R^8 represents H or C_{1-4} alkyl; and

R^9 represents 2-naphthyl, phenyl, C_{1-6} alkoxy or C_{1-8} alkyl (which latter group is optionally substituted by halogen, C_{1-6} alkoxy or C_{1-6} acyloxy);

provided that when R^1 represents R^3, R^3 represents benzyl, methyl, ethyl, *n*-butyl or *n*-hexyl and R^2 represents $C(O)OR^7$, then R^7 does not represent benzyl;

or a pharmaceutically-acceptable salt thereof (hereinafter referred to as "the compounds of the invention").

The compounds of the invention may exhibit tautomerism. All tautomeric forms and mixtures thereof are included within the scope of the invention.

The compounds of the invention may also contain one or more asymmetric carbon atoms and may therefore exhibit optical and/or diastereoisomerism. All diastereoisomers may be separated using conventional techniques, eg chromatography or fractional crystallisation. The various stereoisomers may be isolated by separation of a racemic or other mixture of the compounds using conventional, eg fractional crystallisation or HPLC, techniques. Alternatively the desired optical isomers may be made by reaction of the appropriate optically active starting materials under conditions which will not cause racemisation or epimerisation, or by derivatisation, for example with a homochiral acid followed by separation of the diastereomeric derivatives by conventional means (eg HPLC, chromatography over silica). All stereoisomers are included within the scope of the invention.

According to a further aspect of the invention there is provided the use of a compound of formula I, as hereinbefore defined but without the proviso, as a prodrug.

Alkyl groups which R^3, R^4, R^5, R^6, R^7 and R^9 may represent may be linear or, when there are a sufficient number of carbon atoms, be branched, be cyclic or partially cyclic, be saturated or unsaturated, be interrupted by oxygen and/or be substituted or terminated by OH, provided that the OH group is not attached to an *sp*^{2} carbon atom or a carbon atom which is adjacent to an oxygen atom.

By "partially cyclic alkyl groups" we mean groups such as CH_2Ch.

Alkyl groups which R^8 may represent, and R^3, R^6 and R^7 may be substituted by, may be linear or, when there are a sufficient number of carbon atoms, be branched, be saturated or unsaturated and/or be interrupted by oxygen.

The alkyl portion of alkylphenyl groups which R^3 and R^7 may represent may be linear or, when there are a sufficient number of carbon atoms, be branched and/or be saturated or unsaturated.

Alkylene groups which A^1 may represent may be linear or, when there are a sufficient number of carbon atoms, be branched and/or be saturated or unsaturated.

Alkoxy groups which R^9 may represent, and R^3, R^7 and R^9 may be substituted by, may be linear or, when there are a sufficient number of carbon atoms, be branched and/or be saturated or unsaturated.

Acyloxy groups which R^7 and R^9 may be substituted by may be linear or, when there are a sufficient number of carbon atoms, be branched and/or be saturated or unsaturated.

Abbreviations are listed at the end of this specification.

According to a further aspect of the invention there is provided a compound of formula I, as hereinbefore defined, with the additional provisos that:

(a) R^1 does not represent $-A^1C(O)OR^4$;

(b) R^4 and R^5 do not independently represent H;

(c) R^6 does not represent C_{1-17} alkyl, when R^2 represents $OC(O)R^6$.

According to a further aspect of the invention there is provided a compound of formula I, wherein:

(a) R^1 represents $-A^1C(O)OR^4$;

(b) R^4 and R^5 independently represent H;

(c) R^6 represents C_{1-17} alkyl, when R^2 represents $OC(O)R^6$.

When R^1 represents $-A^1C(O)N(R^4)R^5$, preferred compounds of the invention include those wherein:

A^1 represents C_{1-3} alkylene;

R^4 represents H or C_{1-6} alkyl;

R^5 represents C_{1-6} alkyl or C_{4-6} cycloalkyl; or those wherein

R^4 and R^5 together represent pyrrolidinyl.

When R^1 represents $-A^1C(O)OR^4$, preferred compounds of the invention include those wherein:

A^1 represents C_{1-5} alkylene;

R^4 represents C_{1-6} alkyl.

When R^1 represents R^3, preferred compounds of the invention include those wherein R^3 represents H, C_{1-10} alkyl (which latter group may be linear or, when there are a sufficient number of carbon atoms, may be branched and/or be partially cyclic or cyclic), or C_{1-3} alkylphenyl (which latter groups is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, be branched).

Preferred compounds of the invention include those wherein R^2 represents OH, $OC(O)R^6$ (wherein, in the latter case, R^6 represents optionally substituted phenyl or C_{1-17} alkyl (which latter group may be linear or, when there are a sufficient number of carbon atoms, may be branched, be cyclic or partially cyclic, and/or be saturated or unsaturated)), $C(O)OR^7$ (wherein, in the latter case, R^7 represents optionally substituted phenyl, C_{1-12} alkyl (which latter group is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, may be branched, cyclic or partially cyclic, and/or saturated or unsaturated), or C_{1-3} alkylphenyl (which latter group is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, may be branched)), or $C(O)OCH(R^8)OC(O)R^9$ (wherein, in the latter case, R^8 represents H or methyl, and R^9 represents phenyl, or C_{1-8} alkyl (which latter group is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, may be branched and/or cyclic or partially cyclic)).

More preferred compounds of the invention include those wherein:
R^1 represents H, linear C_{1-10} alkyl, branched C_{3-10} alkyl, partially cyclic C_{4-10} alkyl, C_{4-10} cycloalkyl, optionally substituted linear C_{1-3} alkylphenyl, optionally substituted branched C_3 alkylphenyl, $-A^1C(O)N(R^4)R^5$ (wherein, in the latter case, A^1 represents C_{1-3} alkylene, and R^4 represents H or C_{1-3} alkyl and R^5 represents C_{2-6} alkyl or C_{5-6} cycloalkyl, or R^4 and R^5 together represent pyrrolidinyl), or $-A^1C(O)OR^4$ (wherein, in the latter case, A^1 represents C_{1-5} alkylene and R^4 represents C_{1-4} alkyl);
R^2 represents OH, $OC(O)R^6$ (wherein, in the latter case, R^6 represents

optionally substituted phenyl, linear C_{1-4} alkyl, branched C_{3-4} alkyl or *cis*-oleyl), $C(O)OR^7$ (wherein, in the latter case, R^7 represents optionally substituted and/or optionally unsaturated linear C_{1-4} alkyl or optionally substituted and/or optionally unsaturated branched C_{3-4} alkyl, optionally substituted phenyl, or optionally substituted linear C_{1-3} alkylphenyl or optionally substituted branched C_3 alkylphenyl) or $C(O)OCH(R^8)OC(O)R^9$ (wherein, in the latter case, R^8 represents H or methyl and R^9 represents phenyl, C_{5-7} cycloalkyl, linear C_{1-6} alkyl, branched C_{3-6} alkyl or partially cyclic C_{7-8} alkyl).

Particularly preferred compounds of the invention include those wherein:
R^1 represents linear C_{1-6} alkyl, C_{6-10} cycloalkyl, or optionally substituted linear C_{1-3} alkylphenyl;
R^2 represents OH, $OC(O)R^6$ (wherein, in the latter case, R^6 represents linear C_{1-3} alkyl or branched C_3 alkyl), $C(O)OR^7$ (wherein, in the latter case, R^7 represents optionally substituted linear C_{1-4} alkyl or optionally substituted branched C_{3-4} alkyl, optionally substituted linear C_{1-3} alkylphenyl or branched C_3 alkylphenyl) or $C(O)OCH(R^8)OC(O)R^9$ (wherein, in the latter case, R^8 represents H and R^9 represents C_{5-7} cycloalkyl, linear C_{1-6} alkyl or partially cyclic C_{7-8} alkyl).

When R^1 represents R^3 and R^3 represents optionally substituted C_{1-3} alkylphenyl, preferred optional substituent include C_{1-6} alkyl (especially methyl).

When R^2 represents $C(O)OR^7$ and R^7 represents optionally substituted C_{1-12} alkyl, preferred optional substituents include halogen (especially chloro) and C_{1-6} alkoxy (especially methoxy).

When R^2 represents $C(O)OR^7$ and R^7 represents optionally substituted phenyl, preferred optional substituents include C_{1-6} alkyl (especially methyl), C_{1-6} alkoxy (especially methoxy) and halogen (especially chloro).

When R^2 represents C(O)OR^7 and R^7 represents optionally substituted C_{1-3} alkylphenyl, preferred optional substituents include nitro.

Preferred compounds of the invention include the compounds of Examples 1 to 68.

More preferred compounds of the invention include:

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$;

*n*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$;

*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOEt;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Bu;

PrlC(O)CH$_2$CH$_2$CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

ChNHC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

(*n*Pr)$_2$NC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCC(CH$_3$)$_3$;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCC(CH$_3$)$_3$;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH(CH$_3$)OOCCH$_3$;

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-OOCPh;

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

*n*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

*n*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

*i*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

(*n*Pr)$_2$NCOCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

ChNHCOCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OAc;
HOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;
HOOCCH$_2$-(*R*)Cgl-Aze-Pab-O-*cis*-Oleyl;
Cyclooctyl-OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;
*t*BuCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;
(2-Me)BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;
ChCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;
ChOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;
PhC(Me)$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;
(Me)$_2$CHC(Me)$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;
BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe);
ChCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe);
(2-Me)BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe);
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-Me);
BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-Me);
BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Bu;
*i*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$;
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*i*Bu;
BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Pr;
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCCh;
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCCH$_2$Ch;
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH(Me)OOCPh;
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCPh;
BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH(Me)OAc;
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OAc;
*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OAc;
MeOOC-C(=CHEt)CH$_2$-OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;
Men-OOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe); and

$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2CCl_3$.

Particularly preferred compounds of the invention include:
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2CCl_3$;
$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-COO*n*Bu;
*n*Pr$OOCCH_2$-(*R*)Cgl-Aze-Pab-Z;
Cyclooctyl-$OOCCH_2$-(*R*)Cgl-Aze-Pab-Z;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2OOCCh$;
$MeOOCCH_2$-(*R*)Cgl-Aze-Pab-OH;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-OH;
*n*Pr$OOCCH_2$-(*R*)Cgl-Aze-Pab-OH;
*i*Pr$OOCCH_2$-(*R*)Cgl-Aze-Pab-OH;
$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-OH; and
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-OAc.

Preparation

According to the invention there is also provided a process for the preparation of compounds of formula I which comprises:

(a) Preparation of a compound of formula I wherein R^2 represents OH by reaction of a corresponding compound of formula I, wherein R^2 represents $OC(O)R^6$ and R^6 is as hereinbefore defined, with an alkoxide base (eg an alkali metal alkoxide), for example at room temperature in the presence of an appropriate organic solvent (eg THF).

(b) Preparation of a compound of formula I wherein R^2 represents OH by reaction of a corresponding compound of formula I wherein R^2 represents $C(O)OR^7$ and R^7 is as hereinbefore defined with hydroxylamine, or an acid addition salt thereof, for example at room temperature in the presence of a suitable base (eg potassium carbonate or triethylamine) and an appropriate organic solvent (eg THF or EtOH).

(c) Preparation of a compound of formula I by reaction of a corresponding compound of formula II,

$$\text{H-(}R\text{)Cgl-Aze-Pab-R}^2 \qquad \text{II}$$

wherein R^2 is as hereinbefore defined with a compound of formula III,

$$R^1O(O)C\text{-}CH_2\text{-}L^1 \qquad \text{III}$$

wherein L^1 represents a leaving group, for example halide (eg bromide) or alkylsulphonate (eg trifluoromethylsulphonate) and R^1 is as hereinbefore defined, for example between room and elevated temperature (eg 40°C) in the presence of a suitable base (eg potassium carbonate) and an appropriate organic solvent (eg THF, DMF or acetonitrile).

(d) Preparation of a compound of formula I wherein R^1 represents H and R^2 represents OH or $C(O)OR^7$ and R^7 is as hereinbefore defined by reaction of a corresponding compound of formula I wherein R^1 represents C_{1-10} alkyl or C_{1-3} alkylphenyl, and R^2 represents OH or $C(O)OR^7$, with a suitable base (eg an alkali metal alkoxide or hydroxide), for example at room temperature in the presence of an appropriate organic solvent (eg water or MeOH).

(e) Preparation of a compound of formula I wherein R^2 represents $OC(O)R^6$ and R^6 is as hereinbefore defined, by reaction of a corresponding compound of formula I wherein R^2 represents OH, with a compound of formula IV,

$$R^6C(O)\text{-}O\text{-}C(O)R^6 \qquad \text{IV}$$

or a compound of formula V,

$$R^6C(O)Hal \qquad \text{V}$$

wherein Hal represents Cl or Br and, in both cases, R^6 is as hereinbefore defined, for example at room temperature in the presence of a suitable base (eg triethylamine, pyridine or DMAP) and an appropriate organic solvent (eg methylene chloride or THF).

(f) Preparation of a compound of formula I wherein R^1 represents H and R^2 represents $OC(O)R^6$, and R^6 is as hereinbefore defined, by reaction of a corresponding compound of formula VI

$$P^1O(O)C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}R^2 \qquad \text{VI}$$

wherein P^1 represents an acid labile ester protecting group (eg *t*Bu or Bn), and R^2 represents $OC(O)R^6$, wherein R^6 is as hereinbefore defined, with a suitable acid (eg TFA), for example at room temperature in the presence of an appropriate organic solvent (eg methylene chloride).

(g) Preparation of a compound of formula I wherein R^1 represents R^3, R^3 represents C_{1-10} alkyl or C_{1-3} alkylphenyl, R^2 represents OH or $C(O)OR^7$, and R^7 is as hereinbefore defined, by a trans-esterification of a corresponding compound of formula VII,

$$R^{1a}O(O)C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}R^2 \qquad \text{VII}$$

wherein R^{1a} represents a C_{1-10} alkyl or C_{1-3} alkylphenyl group other than that being formed and R^2 is as hereinbefore defined or an alternative labile alkyl substituent, under conditions which are well known to those skilled in the art.

Compounds of formula II may be prepared by deprotection of a compound of formula VIII,

$$Boc\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}R^2 \qquad \text{VIII}$$

wherein R^2 is as hereinbefore defined, under conditions which are well known to those skilled in the art.

Compounds of formula VI and VII may be prepared analogously to those methods described hereinbefore for preparation of compounds of formula I, in which R^1 represents R^3 and R^3 represents C_{1-10} alkyl or C_{1-3} alkylphenyl.

Compounds of formula VIII may be prepared by reaction of a compound of formula IX,

$$\text{H-Pab-R}^2 \qquad \text{IX}$$

wherein R^2 is as hereinbefore defined with Boc-Cgl-Aze-OH, for example at room temperature in the presence of a suitable coupling system (eg EDC), an appropriate base (eg DMAP) and a suitable organic solvent (eg dichloromethane or acetonitrile).

Compounds of formula VIII, wherein R^2 represents OH may be prepared by reaction of a corresponding compound of formula VIII, wherein R^2 represents $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$, with hydroxylamine, or an acid addition salt thereof, for example at room temperature in the presence of a suitable base (eg potassium carbonate or triethylamine) and an appropriate organic solvent (eg THF or EtOH).

Compounds of formula VIII, wherein R^2 represents $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$, may be prepared by reaction of Boc-(*R*)Cgl-Aze-Pab-H with a compound of formula X,

$$L^2C(O)OR^{2a} \qquad \text{X}$$

wherein L^2 represents a leaving group (eg halogen or phenolate) and R^{2a} represents R^7 or $-CH(R^8)OC(O)R^9$ and R^7, R^8 and R^9 are as hereinbefore defined, for example at or below room temperature in the presence of a suitable base (eg NaOH) and an appropriate organic solvent (eg THF).

Compounds of formula VIII, wherein R^2 represents $OC(O)R^6$ may alternatively be prepared by reaction of a corresponding compound of formula VIII, wherein R^2 represents OH with a compound of formula IV as hereinbefore defined or a compound of formula V as hereinbefore defined, for example at room temperature in the presence of a suitable base (eg triethylamine, pyridine or DMAP) and an appropriate organic solvent (eg methylene chloride or THF).

Compounds of formula VIII wherein R^2 represents $OC(O)R^6$ may alternatively be prepared by reaction of Boc-(*R*)Cgl-Aze-Pab-H with a compound of formula XI,

$$R^6C(O)\text{-}O\text{-}O\text{-}C(O)R^6 \qquad \text{XI}$$

wherein R^6 is as hereinbefore defined, for example at room temperature in the presence of an appropriate organic solvent (eg THF).

Compounds of formula VIII wherein R^2 represents OH may be prepared by reaction of a corresponding compound of formula VIII, wherein R^2 represents $OC(O)R^6$ and R^6 is as hereinbefore defined with a suitable base (eg an alkali metal alkoxide), for example at room temperature in the presence of an appropriate solvent (eg THF).

Compounds of formula IX are well known in the literature or may be prepared using methods analogous to those described hereinbefore. For example, compounds of formula IX wherein R^2 represents $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$ and R^7, R^8 and R^9 are as hereinbefore defined may be prepared by reaction of H-Pab-H, or a protected derivative thereof, with a compound of formula X, as hereinbefore defined, for example at or below room temperature in the presence of a suitable base (eg NaOH) and an appropriate organic solvent (eg THF).

Boc-(*R*)Cgl-Aze-Pab-H may be prepared by reaction of H-Pab-H, or a protected derivative thereof, with Boc-Cgl-Aze-OH, for example as described hereinbefore for compounds of formula VIII.

Boc-(*R*)Cgl-Aze-Pab-H may alternatively be prepared by deprotection of a compound of formula XII,

Boc-(*R*)Cgl-Aze-Pab-P^2 XII

wherein P^2 represents a protecting group orthogonal to Boc, under conditions which are well known to those skilled in the art.

Compounds of formula III, IV, V, X, XI and XII are either commercially available, are well known in the literature, or are available using known techniques (eg as described hereinafter).

The compounds of the invention may be isolated from their reaction mixtures using conventional techniques.

It will be appreciated by those skilled in the art that in the process described above the functional groups of intermediate compounds may need to be protected by protecting groups.

Functional groups which it is desirable to protect include hydroxy, amino, amidino and carboxylic acid. Suitable protecting groups for hydroxy include trialkylsilyl and diarylsilyl groups (eg *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl or trimethylsilyl) and tetrahydropyranyl. Suitable protecting groups for carboxylic acid include C_{1-6} alkyl or benzyl esters. Suitable protecting groups for amino and amidino include *t*-butyloxycarbonyl or benzoyloxy carbonyl. Amidino nitrogens may be either mono or diprotected.

Protecting groups may be removed in accordance with techniques which are well known to those skilled in the art, such as those described hereinafter.

The use of protecting groups is fully described in 'Protective Groups in Organic Chemistry', edited by J W F McOmie, Plenum Press (1973), and 'Protective Groups in Organic Synthesis', 2nd edition, T W Greene & P G M Wutz, Wiley-Interscience (1991).

Medical and pharmaceutical use

The compounds of the invention are useful because they are metabolised in the body to form compounds which possess pharmacological activity. They are therefore indicated as pharmaceuticals and in particular as prodrugs.

In particular, the compounds of the invention, although they are inactive to thrombin *per se*, are metabolised in the body to form potent inhibitors of thrombin, for example as demonstrated in the test described below.

By "the compounds of the invention are inactive to thrombin *per se*" we mean that they exhibit an $IC_{50}TT$ value, as determined in Test A below, of greater than 1 μM.

The compounds of the invention are thus expected to be useful in those conditions where inhibition of thrombin is required.

The compounds of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of thrombosis and hypercoagulability in blood and tissues of animals including man.

It is known that hypercoagulability may lead to thrombo-embolic diseases.

Thrombo-embolic diseases which may be mentioned include activated protein C resistance, such as the factor V-mutation (factor V Leiden), and inherited or acquired deficiencies in antithrombin III, protein C, protein S, heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemi, heparin induced thrombocytopenia and defects in fibrinolysis. The compounds of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions.

The compounds of the invention are further indicated in the treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease.

Particular disease states which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis and pulmonary embolism, arterial thrombosis (eg in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis) and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricle after transmural myocardial infarction.

Moreover, the compounds of the invention are expected to have utility in prophylaxis of re-occlusion (ie thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

In addition to its effects on the coagulation process, thrombin is known to activate a large number of cells (such as neutrophils, fibroblasts, endothelial cells and smooth muscle cells). Therefore, the compounds of the invention may also be useful for the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

Compounds of the invention that inhibit trypsin and/or thrombin may also be useful in the treatment of pancreatitis.

According to a further aspect of the present invention, there is provided a method of treatment of a condition where inhibition of thrombin is required which method comprises administration of a therapeutically effective amount of a compound of formula I as defined above, or a pharmaceutically acceptable salt thereof, to a person suffering from, or susceptible to such a condition.

The compounds of the invention will normally be administered orally, buccally, rectally, dermally, nasally, tracheally, bronchially, by any other parenteral route or *via* inhalation, in the form of pharmaceutical preparations comprising the prodrug either as a free base, or a pharmaceutical acceptable non-toxic organic or inorganic acid addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated and the route of administration, the compositions may be administered at varying doses.

The compounds of the invention may also be combined and/or co-administered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid, ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P_2T) antagonists.

The compounds of the invention may further be combined and/or co-administered with thrombolytics such as tissue plasminogen activator (natural or recombinant), streptokinase, urokinase, prourokinase, anisolated streptokinase plasminogen activator complex (ASPAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction.

According to a further aspect of the invention there is thus provided a pharmaceutical formulation including a compound of formula I as hereinbefore defined, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Suitable daily doses of the compounds of the invention in therapeutical treatment of humans are about 0.001-100mg/kg body weight at peroral administration and 0.001-50mg/kg body weight at parenteral administration.

The compounds of the invention have the advantage that they may have improved pharmacokinetic properties, such as those identified hereinbefore, both after oral and parenteral administration, when compared with compounds of formula:

$$R^{a}O(O)C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}H$$

wherein R^a is as hereinbefore defined, and in particular the compound wherein R^a represents H.

The compounds of the invention are inactive to thrombin, trypsin and other serine proteases. The compounds thus remain inactive in the gastrointestinal tract and the potential complications experienced by orally administered anticoagulants which are active *per se*, such as bleeding and indigestion resulting from inhibition of trypsin, may thus be avoided.

Furthermore, local bleeding associated with and after parenteral administration of an active thrombin inhibitor may be avoided by using the compounds of the invention.

The compounds of the invention may also have the advantage that they may be more efficacious than, be less toxic than, be longer acting than, have a broader range of activity than, produce fewer side effects than, be more easily absorbed than, or that they may have other useful pharmacological properties over, compounds known in the prior art.

Biological Tests

Test A

Determination of Thrombin clotting Time (TT)

Human thrombin (T 6769, Sigma Chem Co, final concentration of 1.4 NIH units/mL) in buffer solution, pH 7.4, 100 μL, and inhibitor solution, 100 μL, were incubated for one min. Pooled normal citrated human plasma, 100 μL, was then added and the clotting time measured in an automatic device (KC 10, Amelung).

The clotting time in seconds was plotted against the inhibitor concentration, and the $IC_{50}TT$ was determined by interpolation.

$IC_{50}TT$ is the concentration of inhibitor that doubles the thrombin clotting time for human plasma.

Test B

Determination of thrombin time in plasma *ex vivo*

The inhibition of thrombin after oral or parenteral administration of the compounds of the invention were examined in conscious rats which, one or two days prior to the experiment, were equipped with a catheter for blood sampling from the carotid artery. On the experimental day, the compound, dissolved in ethanol:Solutol™:water (5:5:90), was administered and blood samples were withdrawn at fixed times into plastic tubes containing 1 part sodium citrate solution (0.13 mol per L.) and 9 parts of blood. The tubes were centrifuged to obtain platelet poor plasma. The plasma was used for determination of thrombin time as described below.

The citrated rat plasma, 100 μL, was diluted with a saline solution, 0.9%, 100 μL, and plasma coagulation was started by the addition of human thrombin (T 6769, Sigma Chem Co, USA) in a buffer solution, pH 7.4, 100 μL. The clotting time was measured in an automatic device (KC 10, Amelumg, Germany).

The concentrations of the active thrombin inhibitor HO(O)C-CH$_2$(*R*)Cgl-Aze-Pab-H (see International Patent Application WO 94/29336) in the rat plasma were estimated by the use of standard curves relating the thrombin time in the pooled citrated rat plasma to known concentrations of the aforementioned active thrombin inhibitor dissolved in saline.

Based on the estimated plasma concentrations of the active thrombin inhibitor HO(O)C-CH$_2$(*R*)Cgl-Aze-Pab-H (which assumes that thrombin time prolongation is caused by the aforementioned compound) in the rat, the area under the curve after oral and/or parenteral administration of the prodrug was calculated (AUCpd) using the trapezoidal rule and extrapolation of data to infinity.

The bioavailability of the active thrombin inhibitor HO(O)C-CH$_2$(*R*)Cgl-Aze-Pab-H after oral or parenteral administration of the prodrug was calculated as below:

[(AUCpd/dose)/(AUCactive,iv/dose] x 100

where AUCactive,iv represents the AUC obtained after intravenous administration of HO(O)C-CH$_2$(*R*)Cgl-Aze-Pab-H to conscious rats as described above.

Test C

Determination of thrombin time in urine *ex vivo*

The amount of the active thrombin inhibitor HO(O)C-CH_2(*R*)Cgl-Aze-Pab-H that was excreted in urine after oral or parenteral administration of the compounds of the invention, dissolved in ethanol:Solutol™:water (5:5:90), was estimated by determination of the thrombin time in urine *ex vivo* (assuming that thrombin time prolongation is caused by the aforementioned compound).

Conscious rats were placed in metabolism cages, allowing separate collection of urine and faeces, for 24 hours following oral administration of compounds of the invention. The thrombin time was determined on the collected urine as described below.

Pooled normal citrated human plasma (100μL) was incubated with the concentrated rat urine, or saline dilutions thereof, for one minute. Plasma coagulation was then initiated by the administration of human thrombin (T 6769, Sigma Chem Company) in buffer solution (pH 7.4; 100μL). The clotting time was measured in an automatic device (KC 10; Amelung).

The concentrations of the active thrombin inhibitor HO(O)C-CH_2(*R*)Cgl-Aze-Pab-H in the rat urine were estimated by the use of standard curves relating the thrombin time in the pooled normal citrated human plasma to known concentrations of the aforementioned active thrombin inhibitor dissolved in concentrated rat urine (or saline dilutions thereof). By multiplying the total rat urine production over the 24 hour period with the estimated mean concentration of the aforementioned active inhibitor in the urine, the amount of the active inhibitor excreted in the urine (AMOUNTpd) could be calculated.

The bioavailability of the active thrombin inhibitor HO(O)C-CH_2(*R*)Cgl-Aze-Pab-H after oral or parenteral administration of the prodrug was calculated as below:

[(AMOUNTpd/dose)/(AMOUNTactive,iv/dose] x 100

where AMOUNTactive,iv represents the amount excreted in the urine after intravenous administration of HO(O)C-CH_2(*R*)Cgl-Aze-Pab-H to conscious rats as described above.

Test D

Determination of HO(O)C-CH_2-(*R*)Cgl-Aze-Pab-H in urine by LC-MS

The amount of the active thrombin inhibitor HO(O)C-CH_2-(*R*)Cgl-Aze-Pab-H that was excreted in urine after oral or parenteral administration of the compounds of the invention, dissolved in ethanol:Solutol™:water (5:5:90), was measured by LC-MS analysis as described below.

The animal studies were performed as described in Method C above. Urine samples were collected and frozen at -20°C before they were analysed.

Urine samples were analysed for their content of HO(O)C-CH_2-(*R*)Cgl-Aze-Pab-H according to the following method:

Thawed urine samples were mixed and, if required, spinned in a centrifuge. Solid phase extraction tubes (Analytichem Bond Elut. No. 1210-2059) were activated with 1.0 mL of methanol and conditioned with 1.0 mL of acetonitrile:water (50:50), followed by 1.0 mL of 0.1% formic acid. 50 μL of the working internal standard (20 μmol/L) was added to each extraction tube. For urine standards, 50 μL of standard solution was added. 200 μL of a sample or, for urine standards, blank urine was added to each tube and thereafter pulled through *via* gravity or a gentle vacuum. Residual urine was washed out with 1.0 mL of ammonium acetate (2 mmol/L), before elution with 1.0 mL of acetonitrile:ammonium acetate (2 mmol/L) (35:65). The collected eluate was transferred to autosampler vials. 30 μL of the extract was injected onto the LC column (Hypersil BDS-C18; 3 μm; 75 mm x 4.0 mm i.d.; Hewlett-Packard No. 79926 03-354), eluted with ammonium acetate buffer (1.3 mmol/L) with 40% acetonitrile and 0.1% formic acid at 0.75 mL/min. The effluent was split so that 30 μL/min entered the electrospray ion source of a P-E Sciex API-3 mass spectrometer. HO(O)C-CH_2-(*R*)Cgl-Aze-Pab-H and HO(O)C-CH_2-(*R*)Cgl-Pro-Pab-H (internal standard) both have retention times near 1.5 minutes. Their molecular ions ($(M+H)^+$) were monitored at m/z 430.2 and 444.2 respectively, at unit mass resolution. Urine standards at two levels, one being at the limit of quantification, were used for calibration based on peak area ratios of HO(O)C-CH_2-(*R*)Cgl-Aze-Pab-H over the internal standard. Linearity of the method was checked over the range 0.050-20 μmol/L. The coefficient of variation was 1-2% at 1-20 μmol/L and 7% at 0.50 μmol/L. The limit of quantification was 0.050 μmol/L.

By multiplying the total urine production over the 24 hour period by the measured concentration of HO(O)C-CH_2-(*R*)Cgl-Aze-Pab-H in the urine, the amount of the active inhibitor excreted in urine (AMOUNTpd) could be calculated. The bioavailability of the active thrombin inhibitor was then calculated as described in Method C above.

The invention is illustrated by way of the following examples.

Examples

General Experimental Procedures.

Mass spectra were recorded on a Finnigan MAT TSQ 700 triple quadrupole mass spectrometer equipped with an electrospray interface.

The ^{1}H NMR and ^{13}C NMR measurements were performed on BRUKER ACP 300 and Varian UNITY plus 400 and 500 spectrometers, operating at ^{1}H frequencies of 300.13, 399.96 and 499.82 MHz respectively, and at ^{13}C frequencies of 75.46, 100.58 and 125.69 MHz respectively. Chemical shifts are reported in δ units.

Preparation of starting materials

Boc-(*R*)Cgl-Aze-Pab-H, Boc-(*R*)Cgl-Aze-Pab x HCl, H-(*R*)Aze-Pab-Z, H-(*R*)Aze-Pab-Z x HCl, Bn-OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z, Boc-(*R*)Cgl-Aze-Pab-Z, Boc-(*R*)Cgl-Aze-OH and Pab-Z x HCl were prepared according to the methods described in International Patent Application WO 94/29336.

Example 1
EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$

(i) Boc-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$

To a solution of Boc-(*R*)Cgl-Aze-Pab-H (6.1 g; 13 mmol) in THF (125 mL) and 2M NaOH (70 mL; 140 mmol) at 0°C was added dropwise allyl chloroformate (1.7 g; 14 mmol). After stirring at 0°C for 1h, the reaction was mixture concentrated, water was added (100 mL) and the resulting aqueous phase was extracted with methylene chloride (3x100 mL). The combined organic phases were concentrated to give 6.4 g of a crude product which was purified by flash chromatography using EtOAc:THF:Et_3N (68:29:3) as eluent. Concentration gave 5.8 g (81%) of the subtitle compound as a white solid.

^1H NMR (500 MHz, $CDCl_3$): δ 8.19 (bt, 1H), 7.78 (d, 2H), 7.26 (d, 2H), 6.02 -5.92 (m, 1H), 5.32 (d, J=17 Hz, 1H), 5.18 (d, J=10 Hz, 1H), 5.06 (d, J=7 Hz, 1H), 4.82 (bs, 1H), 4.61 (d, J=6 Hz, 2H), 4.58-4.48 (m, 1H), 4.38-4.27 (m, 2H), 4.14-4.03 (m, 1H), 3.77-3.68 (m, 1H), 2.60-0.90 (m, 24H).

^{13}C NMR (125 MHz, $CDCl_3$) carbonyl and amidine signals: δ 172.70, 170.74, 168.02, 164.54, 155.98.

(ii) H-(*R*)Cgl-Aze-Pab-$COOCH_2CH=CH_2$ x 2TFA

To a solution of Boc-(*R*)Cgl-Aze-Pab-$COOCH_2CH=CH_2$ (2.03 g; 3.65 mmol; from step (i) above) in methylene chloride (15 mL) at 0°C was added TFA (15 mL). The reaction mixture was stirred at ambient temperature for 3 h followed by concentration to give the 2.8 g of the subtitle compound as a white solid.

^1H NMR (500 MHz, MeOH (d4)): δ 7.80 (d, 2H), 7.57 (d, 2H), 6.02 (m, 1H), 5.45 (d, J=17 Hz, 1H), 5.33 (d, J=10 Hz, 1H), 5.91-4.80 (m, 3H), 4.56 (s, 2H), 4.38 (bq, J=8 Hz, 1H), 3.71 (d, J=7 Hz, 1H), 2.76-2.60 (m, 1H), 2.35-2.20 (m, 1H), 1.9-1.0 (m, 11H).

(iii) $EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2CH=CH_2$

A mixture of H-(*R*)Cgl-Aze-Pab-$COOCH_2CH=CH_2$ x 2TFA (649 mg; 0.95 mmol; from step (ii) above), K_2CO_3 (656 mg, 4.8 mmol), water (0.1 mL), and THF (10 mL) was stirred at 40°C for 2 h followed by addition of ethyl bromoacetate (190 mg; 1.14 mmol) in THF (1 mL). After stirring at 40°C for 4 h and at ambient temperature for 14 h the reaction mixture was filtered, concentrated, and purified by flash chromatography using EtOAc:THF:Et_3N (68:29:3) as eluent to give 244 mg (47%) of the title compound as a white solid.

^1H NMR (400 MHz, $CDCl_3$): δ 8.46 (bt, 1H), 7.81 (d, 2H), 7.35 (d, 2H), 6.08-5.94 (m, 1H), 5.35 (d, J=18 Hz, 1H), 5.23 (d, J=11 Hz, 1H), 4.93 (dd, J=6 and 9 Hz, 1H), 4.66 (d, 2H), 4.62-4.38 (AB part of an ABX-spectrum), 4.16-4.04) (m, 4 H), 3.20 (d, 2H), 2.86 (d, 1H), 2.64-2.45 (m, 2H), 2.0-1.0 (m 17H).

^{13}C NMR (100 MHz, $CDCl_3$) carbonyl and amidine signals: δ 175.33, 172.24, 170.72, 168.19, 164.35.

Example 2

*n*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$

The title compound was prepared according to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$ x 2TFA (503 mg; 0.74 mmol; see Example 1(ii) above) and *n*-propyl bromoacetate (160 mg, 0.88 mmol) to give 277 mg (68%) as a white solid.

^1H-NMR (400 MHz, $CDCl_3$): δ 8.48 (bt, 1H), 7.83 (d, 2H), 7.35 (d, 2H), 6.76 (broad, 1H), 6.02 (m, 1H), 5.37 (dd, 1H), 5.24 (dd, 1H), 4.94 (t, 1H), 4.67 (dd, 2H), 4.49 (AB part of an ABX-spectrum, 2H), 4.12 (m, 2H), 3.98 (t, 2H), 3.24 (AB-system, 2H), 2.87 (d, 1H), 2.52 (m, 2H), 1.99 (bd, 2H), 1.80-1.50 (m, 7H), 1.61 (q, 2H), 1.30-1.10 (m, 2H), 1.00 (qd, 2H), 0.90 (t, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) amidine and carbonyl signals: δ 175.4, 172.3, 170.7, 167.9, 164.5

Example 3

*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$

The title compound was prepared according to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$ x 2TFA (285 mg; 0.42 mmol; see Example 1(ii) above) and *t*-butyl bromoacetate (96 mg; 0.50 mmol) to give 93 mg (39%) as a white solid.

^{1}H NMR (500 MHz, CDCl$_3$): δ 8.50 (bt, 1H), 7.81 (d, 2H), 7.36 (d, 2H), 6.07-5.97 (m, 1H), 5.36 (d, J=16 Hz, 1H), 5.22 (d, J=10 Hz, 1H), 4.93 (dd, J=9 and 6 Hz, 1H), 4.76 (d, J=6 Hz, 2H), 4.57-4.46 (m, 2H), 4.18-4.04 (m, 2H), 3.19-3.08 (AB-spectrum, J_{AB} =20 Hz, 2H), 2.86 (d, J=8 Hz, 1H), 2.72-2.53 (m, 2H), 2.0-0.9 (m, 23H).

^{13}C NMR (100 MHz, CDCl$_3$) amidine and carbonyl signals: δ 175.28, 171.53, 170.76, 167.81, 164.1.

Example 4

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOEt

(i) Boc-(*R*)Cgl-Aze-Pab-COOEt

The sub-title compound was prepared according the procedure described in Example 1(i) from Boc-(*R*)Cgl-Aze-Pab-H (600 mg; 1.3 mmol) and ethyl chloroformate (150 mg; 1.4 mmol) yielding 240 mg (34%) as a white solid.

^{1}H-NMR (300 MHz, CDCl$_3$): δ 9.37 (bs, 1H), 8.16 (bs, 1H), 7.72 (d, 2H), 7.18 (d, 2H), 5.17 (d, 1H), 4.73 (t, 1H), 4.47 (dd, 1H), 4.27 (m, 2H), 4.06 (q, 2H), 3.66 (t, 1H), 2.48 (m, 1H), 2.37 (m, 1H), 1.4-1.8 (m, 7H), 1.22 (s, 9H), 1.3-0.8 (m, 7H).

^{13}C-NMR (75MHz, CDCl$_3$) carbonyl and amidine signals: δ 172.6, 170.7, 167.9, 164.8, 156.0

(ii) H-(*R*)Cgl-Aze-Pab-COOEt x 2HCl

To a solution of Boc-(*R*)Cgl-Aze-Pab-COOEt (240 mg; 0.44 mmol; from step (i) above) in EtOAc (20 mL) was added hydrogen chloride at 0°C over 5 minutes. The reaction mixture was stirred at 0°C for 1 h followed by concentration to give 225 mg (100%) as a white solid.

^{1}H-NMR (300 MHz, D_2O): δ 7.85 (d, 2H), 7.61 (d, 2H), 4.98 (dd, 1H), 4.60 (s, 1H), 4.44 (p, 5H), 3.90 (d, 1H), 2.73 (m, 1H), 2.37 (m, 1H), 2.0-1.65 (m, 9H), 1.39 (t, 3H), 1.4-1.1 (m, 7H), 0.98 (m, 1H).
^{13}C-NMR (75 MHz, D_2O) amidine and carbonyl signals: δ 172.7, 169.4, 166.8, 154.3.

(iii) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOEt

The title compound was prepared according to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOEt x 2HCl (160 mg; 0.31 mmol; from step (ii) above) and ethyl bromoacetate (52.5 mg; 0.31 mmol). Yield: 100 mg (61%) as a light yellow powder.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.48 (bt, 1H), 7.81 (d, 2H), 7.38 (d, 2H), 4.51 (AB part of an ABX-spectrum, 2H), 4.21 (q, 2H), 4.15-4.05 (m, 4H), 3.21 (AB-spectrum, 2H), 2.86 (d, 1H), 2.68 (m, 1H), 2.53 (m, 1H), 1.96 (bd, 2H), 1.90-1.70 (m, 12H), 1.35 (t, 3H), 1.22 (t, 6H), 1.30-0.95 (m, 2H).
^{13}C-NMR (75 MHz, $CDCl_3$) carbonyl and amidine signals: δ 175.5, 172.2, 170.7, 167.6, 164.9

Example 5

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Pr

(i) Boc-(*R*)Cgl-Aze-Pab-COO-*n*Pr

The sub-title compound was prepared according to the procedure described in Example 1(i) above using Boc-(*R*)Cgl-Aze-Pab-H (6.0 g; 13 mmol;) and *n*-propyl chloroformate (1.57 mL; 14 mmol). Yield 5.4 g (76%).

^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.25 (bt, 1H), 7.82 (d, 2H), 7.31 (d, 2H), 5.09 (bd, 1H), 4.87 (dd, 1H), 4.58 (dd, 1H), 4.39 (dd, 2H), 4.14 (q, 1H), 4.10 (t, 2H), 3.79 (t, 1H), 2.54 (dm, 2H), 2.21 (s, 1H), 1.87-1.55 (m, 8H), 1.33 (s, 9H), 1.45-1.0 (m, 4H), 0.99 (t, 3H).
^{13}C-NMR (100 MHz, CDCl$_3$) amidine and carbonyl signals: δ 172.7, 170.6, 167.8, 165.0, 155.9.

(ii) H-(*R*)Cgl-Aze-Pab-COO-*n*Pr x 2TFA

The sub-title compound was prepared according to the procedure described in Example 1(ii) using 2.1 g (3.7 mmol) of Boc-(*R*)Cgl-Aze-Pab-COO-*n*Pr (from step (i) above). Yield 3.7 g.

^{1}H-NMR (400 MHz, MeOH-d$_4$): δ 7.77 (d, 2H), 7.60 (d, 1H), 4.86 (dd, 1H), 4.56 (AB part of an ABX-spectrum, 2H), 4.33 (m, 4H), 3.72 (d, 1H), 3.30 (m, 1H), 2.68 (m, 1H), 2.28 (m, 1H), 1.9-1.7 (m, 9H), 1.4-1.1 (m, 6H), 1.02 (t, 3H).
^{13}C-NMR (100 MHz, MeOH-d$_4$) carbonyl and amidine signals: δ 172.7, 169.3, 168.0, 161.4.

(iii) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Pr

The title compound was prepared according to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-*n*Pr x 2TFA (472 mg; 0.69

mmol; from step (ii) above) and ethyl bromoacetate (138 mg; 0.83 mmol) to give 0.22 mg (58%) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.46 (bt, 1H), 7.82 (d, 2H), 7.32 (d, 2H), 4.92 (dd, 1H), 4.49 (AB part of an ABX-spectrum, 2H), 4.10 (m, 6H), 3.23 (AB-spectrum, 2H), 2.80 (dm, 2H), 1.98 (bd, 2H),1.74 (q, 2H), 1.63 (dd, 2H), 1.52 (m, 1), 1.21 (t, 3H), 1.20-1.10 (m, 2H), 0.98 (t, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) carbonyl and amidine signals: δ 175.3, 172.2, 170.7, 167.6, 164.8.

Example 6

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Pr

The title compound was prepared according to the procedure described in Example 1(iii) above from H-(*R*)Cgl-Aze-Pab-COO-*n*Pr x 2TFA (365 mg; 0.53 mmol; see Example 5(ii) above) and methyl bromoacetate (98 mg; 0.64 mmol) to give 114 mg (41%) as a white solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.44 (bt, 1H), 7.82 (d, 2H), 7.32 ((d, 2H), 7.04 (broad, 1H), 4.92 (dd, 1H), 4.49 (AB part of an ABX spectrum), 4.12 (m, 2H), 4.10 (t, 2H), 3.63 (s, 3H), 3.24 (s, 2H), 2.87 (d, 1H), 2.65 (m, 1H), 2.52 (m, 1H), 2.01 (broad, 1H), 1.96 (bd, 2H), 1.75 (q, 4H), 1.63 (bdd, 1H), 1.53 (m, 1H), 1.3-1.1 (m, 5H), 0.99 (t, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) carbonyl and amidine signals: δ 175.3, 172.5, 170.7, 167.7, 165.0.

Example 7

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH$_2$OMe

(i) Boc-(*R*)Cgl-Aze-Pab-COOCH$_2$CH$_2$OMe

The sub-title compound was prepared according to the procedure described in Example 1(i) above using Boc-(*R*)Cgl-Aze-Pab-H (6.0 g; 13 mmol) and 2-methoxyethyl chloroformate (1.94 g; 14 mmol). Yield 3.9 g (52%).

^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.24 (bt, 1H), 7.83 (d, 2H), 7.31 (d, 2H), 5.08 (bd, 1H), 4.87 (dd, 1H), 4.58 (dd, 1H), 4.39 (dd, 2H), 4.30 (t, 2H), 4.15 (m, 1H), 3.79 (bt, 1H), 3.68 (t, 2H), 3.40 (s, 3H), 2.65-2.45 (m, 2H), 2.20 (broad, 1H), 1.9-1.55 (m, 6H), 1.34 (s, 9H), 1.3-0.95 (m, 6H).
^{13}C-NMR (100 MHz, CDCl$_3$) carbonyl and amidine signals: δ 172.7, 170.7, 167.8, 164.6, 155.9.

(ii) H-(*R*)Cgl-Aze-Pab-COOCH$_2$CH$_2$OMe x 2TFA

The sub-title compound was prepared according to the procedure described in Example 1(ii) above using 1.71 g of Boc-(*R*)Cgl-Aze-Pab-COOCH$_2$CH$_2$OMe (from step (i) above). Yield 1.89 g (88%).

^{1}H-NMR (400 MHz, MeOH-d4): δ 7.77 (d, 2H), 7.59 (d, 2H), 4.85 (dd, 1H), 4.56 (d, 2H), 4.49 (m, 2H), 4.37 (m, 1H), 4.28 (m, 1H), 3.70 (m, 3H), 3.37 (s, 3H), 2.68 (m, 1H), 2.28 (m, 1H), 1.9-1.7 (m, 7H), 1.4-1.1 (m, 6H).
^{13}C-NMR (100 MHz, MeOH-d4) carbonyl and amidine signals: δ 172.7, 169.3, 168.0, 154.6.

(iii) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH$_2$OMe

The title compound was prepared according to the procedure described in Example 1(iii) above from H-(*R*)Cgl-Aze-Pab-COOCH$_2$CH$_2$OMe x 2TFA

(487 mg; 0.69 mmol; from step (ii) above) and ethyl bromoacetate (138 mg; 0.83 mmol) to give a crude product which was purified by flash chromatography using THF:methylene chloride (3:1) as eluent. The yield was 0.13 mg (34 %) as a white solid.

^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.46 (bt, 1H), 7.83 (d, 2H), 7.32 (d, 2H), 7.21 (broad, 1H), 4.92 (dd, 1H), 4.49 (AB part of an ABX spectrum, 2H), 4.30 (t, 2H), 4.12 (q, 2H), 4.07 (q, 2H), 3.68 (t, 1H), 3.40 (s, 3H), 3.24 (s, 2H), 2.62 (m, 1H), 2.52 (m, 1H), 2.07 (broad, 1H), 1.97 (bd, 1H), 1.8-1.5 (m, 5H), 1.3-1.1 (m, 6H), 1.05-0.95 (m, 2H).
^{13}C-NMR (100 MHz, CDCl$_3$) carbonyl and amidine signals: δ 175.3, 172.2, 170.7, 167.8, 164.6.

Example 8

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH$_2$OMe

The title compound was prepared according to the method described in Example 1(iii) above from H-(*R*)Cgl-Aze-Pab-COOCH$_2$CH$_2$OMe x 2TFA (490 mg; 0.7 mmol; see Example 7(ii) above) and methyl bromoacetate (128 mg; 0.84 mmol) to give a crude product which was purified by flash chromatography using THF:methylene chloride (3:1) as eluent. The yield was 155 mg (41 %) as a white solid.

^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.44 (t, 1H), 7.83 (d, 2H), 7.31 (d, 2H), 4.92 (dd, 1H), 4.49 (AB part of an ABX spectrum, 2H), 4.30 (t, 2H), 4.13 (m, 2H), 3.68 (t, 2H), 3.63 (s, 3H), 3.39 (s, 3H), 3.25 (s, 2H), 2.87 (d, 1H), 2.62 (m, 1H), 2.52 (m, 1H), 1.96 (bd, 1H), 1.8-1.5 (m, 6H), 1.3-1.1 (m, 5H), 1.00 (q, 2H).
^{13}C-NMR (100 MHz, CDCl$_3$) carbonyl and amidine signals: δ 175.2, 172.6, 170.7, 167.8, 164.5.

Example 9

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Bu

(i) Boc-(*R*)Cgl-Aze-Pab-COO-*n*Bu

The sub-title compound was prepared according to the procedure described in Example 1(i) from Boc-(*R*)Cgl-Aze-Pab-H (1.01 g; 2.1 mmol) and *n*-butyl chloroformate (0.32 g; 2.4 mmol). After stirring at ambient temperature for 1.5 h the reaction mixture was concentrated and extracted with three portions of methylene chloride. The combined organic phase was then washed with water, dried over Na_2SO_4, and concentrated to give 1.0 g (83%) of the sub-title compound as a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.81-9.31 (bs, 1H), 8.36-8.20 (m, 1H), 7.35 (d, 2H), 7.84 (d, 2H), 6.78-6.43 (bs, 1H), 5.05-4.82 (m, 2H), 4.69-4.15 (m, 3H), 4.15-4.08 (m, 3H), 3.86-3.70 (m, 1H), 2.68-2.42 (m, 2H), 1.92-0.88 (m, 25H).

^{13}C-NMR (125 MHz, $CDCl_3$) amidine and carbonyl signals: δ 172.5, 170.7, 167.9, 164.9, 156.0.

FAB-MS: (m+1)=572 (m/z)

(ii) H-(*R*)Cgl-Aze-Pab-COO-*n*Bu x 2HCl

The sub-title compound was prepared according to the procedure described in Example 4(ii) from Boc-(*R*)Cgl-Aze-Pab-COO-*n*Bu (2.5 g; 4.4 mmol; from step (i) above) to give 2.4 g (100%) as a white solid.

^{1}H-NMR (300 MHz, MeOH-d4): δ 7.78-7.60 (m, 2H), 4.66 -4.49 (m, 2H), 0.98 (t, 2H), 4.49-4.35 (m, 3H), 4.35-4.22 (m, 1H), 3.75 (d, 1H), 1.92-1.67 (m, 8H), 1.56-1.07 (m, 8H). The signal of one of the protons is partially obscured by the CD_3OH-signal

^{13}C-NMR (100 MHz, MeOH-d4) amidine and carbonyl signals: δ 172.7, 169.3, 167.9, 154.7

MS (m+1)=472 (m/z)

(iii) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Bu

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-*n*Bu x 2HCl (400 mg; 0.74 mmol) and ethyl bromoacetate (147 mg; 0.88 mmol). The product was purified by flash chromatography using methylene chloride and EtOH gradient 0.1% = > 12.8% as eluent to give 290 mg (70%) as a white solid.

^{1}H-NMR (300MHz, CDCl$_3$): δ 9.70-9.36 (bs, 1H), 8.47 (t, 1H), 7.81 (d, 2H), 7.32 (d, 2H), 7.07-6.73 (bs, 1H), 4.97-4.87 (dd, 1H), 4.62-4.35 (m, 2H), 4.20-3.98 (m, 6H), 3.27-3.12 (m, 2H), 2.84 (s, 1H), 2.70-2.40 (m, 2H), 2.03-0.85 (m, 22H)

^{13}C-NMR (75MHz, CDCl$_3$) amidine and carbonyl signals: δ 175.3, 172.3, 170.8, 167.9, 165.0

FAB-MS: (m+1)=558 (m/z)

Example 10

PrlC(O)CH$_2$CH$_2$CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

(i) PrlC(O)CH$_2$CH$_2$CH$_2$OH

A mixture of γ-butyrolactone (4.0 g; 46.5 mmol) and pyrrolidine (6.6 g; 92.8 mmol) was stirred at room temperature for 2.5 h. The product was concentrated in vacuum to give 14.5 g (100%) of the product as a yellow oil.

^{1}H-NMR (300MHz, MeOH-d4): δ 3.58 (t, 2H), 3.50 (t, 2H), 3.40 (t, 2H), 2.42 (t, 2H), 2.06-1.75 (m, 6H)

(ii) PrlC(O)$CH_2CH_2CH_2$OOCCH_2Br

To a mixture of PrlC(O)$CH_2CH_2CH_2$OH (7.2 g; 45.8 mmol; from step (i) above) and DMAP (5.6 g; 45.8 mmol) in methylene chloride at 0°C was added dropwise bromoacetyl bromide (4.0 mL; 45.8 mmol). After stirring at room temperature for 1.5 h another portion of bromoacetyl bromide (1.0 mL, 11.4 mmol) and DMAP (1.4 g, 11.4 mmol) was added and reaction was refluxed for 1.5 h. Water was added and the methylene chloride was extracted 3 times. The organic phase was dried with Na_2SO_4 and concentrated to give 10.3 g (81%) of the product as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.15 (t, 2H), 3.75 (s, 2H), 3.40-3.31 (m, 4H), 2.30 (t, 2H), 1.98-1.83 (m, 4H), 1.81 -1.73 (m, 2H)

(iii) PrlC(O)$CH_2CH_2CH_2$OOCCH_2-(*R*)Cgl-Aze-Pab(Z)

The title compound was prepared according to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z (6 g; 10.4 mmol) and PrlC(O)$CH_2CH_2CH_2$OOCCH_2Br (3.5 g; 12.4 mmol; from step (ii) above). The crude product was purified by flash chromatography using heptane:EtOAc:isopropanol (1:2:2) as eluent to give 4.2 g which was then purified by using preparative RPLC using 44% acetonitrile in 0.1M NH_4OAc as eluent to give 2.64 g (36%) of the product as a white solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.80- 9.22 (b s, 1H), 8.36 (t, 1H), 7.96 -7.58 (m, 3H), 7.45 (d, 2H), 7.37 -7.22 (m, 5H), 5.20 (s, 2H), 4.95 - 4.88 (dd, 1H), 4.72 -4.29 (m, 2H), 4.15 -4.04 (m, 2H), 4.04 -3.88 (m, 2H), 3.40 (t, 2H), 3.34 (t, 2H), 3.28 -3.17 (m, 2H), 2.85 (d, 1H), 2.67 -2.48 (m, 1H), 2.23 (t, 2H), 2.14 -0.93 (m, 18H).

^{13}C-NMR (125 MHz, $CDCl_3$) amidine and carbonyl signals: δ 175.3, 172.4, 170.9, 170.4, 168.2, 164.6

FAB-MS: (m+1)=703 (m/z)

Example 11

ChNHC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

(i) ChNHC(O)CH$_2$OH

A mixture of cyclohexylamine (9.9 g; 99.8 mmol) and 2,5-dioxo-1,4-dioxane (3.0 g, 25.9 mmol) was stirred at 100°C for 2.5 h. The product was concentrated to give 8.1 g (100%) of the product as a brown solid.

^{1}H-NMR (500 MHz, MeOH-d4): δ 3.92 (s, 2H), 3.75 -3.65 (m, 1H), 1.90 -1.58 (m, 5H), 1.43 -1.07 (m, 5H). The signal of two of the protons are obscured by the CD$_3$OH-signal.

^{13}C-NMR (125 MHz, MeOH-d4) amidine and carbonyl signals: δ 174.0, 62.5, 33.7, 26.5, 26.1, 26.0 The signal of one of the carbons is obscured by the CD$_3$OD-signal.

(ii) ChNHC(O)CH$_2$OOCCH$_2$Br

To a mixture of ChNHC(O)CH$_2$OH (8.0 g; 50.9 mmol; from step (i) above) and DMAP (6.2 g; 50.9 mmol) in methylene chloride (80 mL) at 0°C was added dropwise bromoacetyl bromide (4.0 mL; 45.8 mmol). After stirring at room temperature for 1.5 h further portions of bromoacetyl bromide (1.0 mL, 11.4 mmol) and DMAP (1.4 g, 11.4 mmol) were added and the reaction mixture was refluxed for 1.5 h. Water was added and the aqueous phase was extracted with three portions of methylene chloride. The organic phase was washed with water, dried with Na$_2$SO$_4$, and concentrated to give 10.3 g (73%) of the product as a brown solid.

^{1}H NMR (400 MHz, CDCl$_3$): δ 6.12-6.00 (bs, 1H), 4.62 (s, 2H), 3.90 (s, 2H), 3.84-3.76 (m, 1H), 1.95-1.86 (m, 2H), 1.75-1.65 (m, 2H), 1.65 - 1.56 (m, 1H), 1.43-1.29 (m, 2H), 1.24-1.10 (m, 3H).

(iii) ChNHC(O)CH_2OOCCH_2-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) with starting from H-(*R*)Cgl-Aze-Pab-Z (6 g; 10.4 mmol) and ChNHC(O)CH_2OOCCH_2Br (3.5 g; 12.4 mmol; from step (ii) above). The crude product was purified by flash chromatography using heptane:EtOAc:isopropanol (5:2:2) as eluent followed by concentration and then by preparative RPLC using 50% acetonitrile in 0.1M NH_4OAc as eluent. Concentration and freeze drying gave 2.6 g (36%) of the product as a white solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.78-9.25 (bs, 1H), 7.90 (t, 1H), 7.78 (d, 2H), 7.44 (d, 2H), 7.38-7.24 (m, 5H), 6.66 (t, 1H), 5.20 (s, 2H), 4.90-4.83 (dd, 1H), 4.60-4.45 (m, 2H), 4.18-3.93 (m, 4H), 3.73-3.62 (m, 1H), (d, 1H), 3.23, 3.44 (AB, 2H), 2.87, 2.65-2.08 (m, 3H), 1.98-0.93 (m, 22H)

^{13}C-NMR (125 MHz, $CDCl_3$) amidine and carbonyl signals: δ 175.1, 171.7, 170.7, 168.8, 166.1, 164.4

FAB-MS: (m+1)=703 (m/z)

Example 12

$(nPr)_2$NC(O)CH_2OOCCH_2-(*R*)Cgl-Aze-Pab-COOCH_2OOCC$(CH_3)_3$

(i) $(nPr)_2$NC(O)CH_2OH

A mixture of 2,5-dioxo-1,4-dioxane (2.02 g; 17.4 mmol) and di-*n*-propylamine (5 ml; 36.5 mmol) was heated at 50°C for 1 h and at 90°C for 66 h. Toluene was added and subsequently removed in vacuo with excess di-*n*-propylamine. The residue was purified by flash chromatography using 10% methanol in methylene chloride as eluent to give 4.18 g (66%) of the desired compound.

^{1}H NMR (300 MHz, CDCl3): δ 4.1 (d, 2 H), 3.65 (t, 1 H), 3.25-3.35 (m, 2 H), 2.9-3.0 (m, 2 H), 1.45-1.6 (m, 4 H), 0.8-0.95 (m, 6 H)

(ii) $(n\mathrm{Pr})_2\mathrm{NC(O)CH_2OOCCH_2Br}$

A mixture of $(n\mathrm{Pr})_2\mathrm{NC(O)CH_2OH}$ (0.743 g; 4.7 mmol; from step (i) above), DCC (0.951 g, 4.6 mmol), and bromoacetic acid (0.704 g; 5.1 mmol) in methylene chloride (15 ml) was stirred at room temperature for 1.5 h. The precipitate was removed by filtration and the solvent was removed from the filtrate in vacuo. Kugelrohr distillation of the residue gave 0.66 g (50%) of the desired compound.

^{1}H NMR (300 MHz, CDCl3): δ 4.8 (s, 2 H), 4.0 (s, 2 H), 3.2-3.3 (m, 2 H), 3.05-3.15 (m, 2 H), 1.5-1.7 (m, 4 H), 0.8-1.0 (dt, 6 H)

(iii) Pivaloyloxymethyl 4-nitrophenyl carbonate

A mixture of silver pivalate (7.5 g; 25 mmol) and iodomethyl 4-nitrophenyl carbonate (Alexander *et al*, J. Med. Chem. (1988) 31, 318; 7.99 g; 25 mmol) was refluxed in benzene (50 ml) for 2 h. The benzene was removed in vacuo and the residue was dissolved in toluene. Filtration through hyflo and purification by flash chromatography using toluene as eluent afforded 4.00 g (54%) of the sub-title compound.

^{1}H NMR (300 MHz; CDCl$_3$): δ 8.25 (d, 2 H), 7.40 (d, 2 H), 5.85 (s, 2 H), 1.2 (s, 2 H)
^{13}C NMR (75 MHz, CDCl$_3$) amidine and carbonyl signals: δ 176.77, 155.06

(iv) Boc-(*R*)Cgl-Aze-Pab-$\mathrm{COOCH_2OOCC(CH_3)_3}$

A solution of pivalyloxymethyl 4-nitrophenyl carbonate (1.18 g; 4 mmol; from step (iii) above) in methylene chloride (20 ml) was added at room temperature to a solution of Boc-(*R*)Cgl-Aze-Pab-H (1.88g; 4 mmol) and triethylamine (0.66 ml; 4.75 mmol) in methylene chloride (20 ml). After 1 h the methylene chloride was replaced by EtOAc and the mixture was purified by flash chromatography using EtOAc as eluent to give 1.27 g (50%) of sub-title compound.

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.5 (bs, 1 H), 8.25 (t, 1 H), 7.8 (d, 2 H), 7.3 (d, 2 H), 7.0 (bs, 1 H), 5.0-4.8 (m, 2 H), 4.65-4.5 (m, 1 H), 4.5-4.3 (m, 2 H), 4.2-4.05 (m, 1 H), 3.75 (t, 1 H), 2.7-2.4 (m, 2 H), 1.9 -1.45 (m, 5 H), 1.45 - 0.8 (m, 24 H)

(v) H-(*R*)Cgl-Aze-Pab-$COOCH_2OOCC(CH_3)_3$

Boc-(*R*)Cgl-Aze-Pab-$COOCH_2OOCC(CH_3)_3$ (327 mg; 0.52 mmol; from step (iv) above) was dissolved in a mixture of methylene chloride (5 ml) and TFA (1.2 ml). After 2 h the reaction mixture was concentrated in vacuo, acetonitrile was added, and the solvent was again removed in vacuo to give crude sub-title product which was used without further purification in the next step.

(vi) $(nPr)_2NC(O)CH_2OOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2OOCC(CH_3)_3$

The residue from step (v) above was mixed with $(nPr)_2NC(O)CH_2OOCCH_2Br$ (150 mg; 0.53 mmol; from step (ii) above) and K_2CO_3 (480 mg; 3.5 mmol) in THF (5 ml) and heated for 3 h at 40°C. The reaction mixture was filtered and concentrated to a crude product which was purified by preparative RPLC to give 78 mg (21%) of the title compound.

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.3-9.6 (bs, 1 H), 8.5 (m, 1 H), 7.95-8.15 (bs, 1 H), 7.85-7.95 (d, 2 H), 7.2-7.3 (d, 2 H), 5.8 (s, 2 H), 4.8-4.9 (dd, 1 H), 4.5-4.7 (m, 3 H), 4.0-4.4 (m, 3 H), 2.8-3.4 (m, 5 H), 2.2-2.7

(m, 3 H), 1.75-1.3 (m, 9 H), 1.3-1.0 (m, 14 H), 1.0-0.7 (m, 7 H).
^{13}C NMR (75 MHz, CDCl3) amidine and carbonyl signals: δ 177.24, 175.30, 171.85, 170.79, 168.78, 165.82, 163.14.

Example 13

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCC(CH$_3$)$_3$

The title compound was prepared analogously to to the procedure described in Example 12(vi) above from crude Boc-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCC(CH$_3$)$_3$ (0.41 g; 0.65 mmol; see Example 12(iv) above) using acetonitrile (10 ml) as solvent. After stirring over night at room temperature, the solvent was removed *in vacuo* and the residue was partitioned between EtOAc and water. The aqueous phase was extracted three times with EtOAc and the combined organic phases were dried (Na$_2$SO$_4$) and the solvent was removed *in vacuo*. The residue was subjected to flash chromatography using methylene chloride/methanol as eluent. Freeze drying from glacial acetic acid gave 84 mg (21%) of the title compound.

^{1}H NMR (300 MHz, CDCl3): δ 9.9 (bs, 1 H), 8.5 (t, 1 H), 7.35 (d, 2 H), 5.85 (s, 2 H), 5.90 (dd, 2 H), 4.6-4.35 (m, 2 H), 4.15-4.0 (m, 4), 3.2 (s, 2 H), 2.85 (d, 1 H), 2.7-2.45 (m, 2), 2.0-1.9 (m, 2 H), 1.8-1.45 (m, 5 H), 1.3-0.9 (m, 18 H).
^{13}C NMR (75 MHz, CDCl3) amidine and carbonyl signals: δ 177.23, 175.48, 172.29, 170.80, 168.85, 163.14.

Example 14

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH(CH$_3$)OOCCH$_3$

(i) Boc-(*R*)Cgl-Aze-Pab-COOCH(CH$_3$)OOCCH$_3$

A solution of Boc-(*R*)Cgl-Aze-Pab-H (6.38 g; 13.5 mmol), 1-acetoxyethyl

4-nitrophenyl carbonate (Alexander *et al*, J. Med. Chem. (1988) 31, 318) (3.05 g; 12 mmol), and triethylamine (1.95 ml; 14 mmol) in methylene chloride (40 ml) was stirred at room temperature for 16 h followed by addition of EtOAc. The resulting solution was slightly concentrated and washed with aqueous Na_2CO_3 (10%), concentrated to a crude product, which was purified by flash chromatography using EtOAc as eluent, to give 5.59 g (77%) of the sub-title compound.

^{1}H NMR (300 MHz, CDCl3): δ 9.5 (bs, 1 H), 8.25 (t, 1 H), 7.85 (d, 2 H), 7.35 (d, 2 H), 6.95 (q, 1 H), 6.7 (bs, 1 H), 5.0-4.85 (m, 2 H), 4.65-4.5 (m, 1 H), 4.5-4.25 (m, 2 H), 4.2-4.05 (m, 1 H)3.75 (t, 1 H), 2.65-2.45 (m, 2 H)2.05 (s, 3 H), 1.9-1.45 (m, 11 H), 1.45-0.8 (m, 12 H).
^{13}C NMR (75 MHz, CDCl3) amidine and carbonyl signals: δ 172.61, 170.80, 169.54, 168.91, 162.50, 156.02.

(ii) H-(*R*)Cgl-Aze-Pab-COOCH(CH$_3$)OOCCH$_3$

The crude sub-title compound was prepared according to the procedure described in Example 12(v) above from Boc-(*R*)Cgl-Aze-Pab-COOCH(CH_3)OOCCH_3 (2.21 g; 3.68 mmol; from step (i) above).

(iii) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH(CH$_3$)OOCCH$_3$

The crude H-(*R*)Cgl-Aze-Pab-COOCH(CH_3)OOCCH_3 from step (ii) above was dissolved in methylene chloride (150 mL). The mixture was washed with a 10% Na_2CO_3 solution and the organic phase was dried with K_2CO_3 and filtered. To the resulting solution as added K_2CO_3 (756 mg, 5.5 mmol) and ethyl (O-trifluoromethanesulphonyl)-glycolate (790 mg; 3.3 mmol) in methylene chloride (5 ml). The reaction mixture was stirred for 5-10 minutes at room temperature and then concentrated in vacuo. The residue was dissolved in EtOAc and the resulting mixture was filtered through celite. The filtrate was subjected to flash chromatography using

EtOAc as eluent followed by HPLC to give 475 mg (22%) of the title compound.

^{1}H NMR (300 MHz, CDCl3): δ 9.5 (bs, 1 H), 8.3 (t, 1 H), 7.7 (d, 2 H), 7.2 (d, 2 H), 6.85 (q, 1 H), 4.8 (t, 1 H), 4.45-4.25 (m, 2 H), 4.1-3.85 (m, 4 H), 3.1 (s, 2 H), 2.75 (s, 1 H), 2.5-2.3 (m, 2 H),1.95 (s, 3 H), 1.9-1.8 (m, 1 H), 1.7-1.25 (m, 8 H), 1.25-1.75 (m, 8 H).
^{13}C NMR (75.5 MHz, CDCl3) amidine and carbonyl signals: δ 175.26, 172.34, 170.81, 169.49, 168.80, 162.43.

Example 15

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-OOCPh

(i) Boc-(*R*)Cgl-Aze-Pab-OOCPh

To a solution of Boc-(*R*)Cgl-Aze-Pab-H (1.0 g; 2.1 mmol) and Na_2HPO_4 (18.7 g; 105 mmol) in THF (45 mL) at 20° C was added dropwise dibenzoyl peroxide (556 mg; 2.3 mmol) dissolved in THF (10 mL) over 45 minutes. After stirring at 20°C for 24 h, the reaction mixture was concentrated and the resulting crude product was subjected to preparative RPLC. This gave 124 mg (10%) of the sub-title compound as a white solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.26 (m, 1H), 8.09 (m, 2H), 7.72 (m, 2H), 7.59 (m, 1H), 7.48 (m, 2H), 7.36 (d, 2H), 5.13 (s, 2H), 4.87-4.98 (m, 2H), 4.54-4.61 (m, 1H), 4.33-4.47 (m, 2H), 4.13-4.19 (m, 1H), 3.81 (t, 1H), 2.53-2.63 (m, 2H), 1.73-1.86 (m, 3H), 1.66-1.72 (m, 2H), 1.36 (s, 9H), 0.968-1.28 (m, 6H).
^{13}C-NMR (100 MHz, $CDCl_3$) amidine and carbonyl signals: δ 172.7, 170.6, 163.9, 157.0, 155.9.
LC-MS: m/z 592 ($M+H^+$); m/z 614 ($M+Na^+$).

(ii) H-(*R*)Cgl-Aze-Pab-OOCPh

To a solution of Boc-(*R*)Cgl-Aze-Pab-OOCPh (600 mg; 1.01 mmol; from step (i) above) in methylene chloride (18 mL) was added TFA (6 mL) at 20°C. After stirring for 14 h, the reaction mixture was concentrated and the resulting crude product was partitioned between EtOAc:0.1 M NaOH. The phases were separated and the organic layer was dried (Na_2SO_4) and evaporated. Yield: 480 mg (96%) as a white solid.

^{1}H-NMR (400 MHz, MeOH-d4): δ 8.18 (m, 2H), 7.77 (m, 2H), 7.64 (m, 1H), 7.52 (m, 2H), 7.43 (d, 2H), 4.75-4.81 (m, 1H), 4.50 (s, 2H), 4.18-4.34 (m, 2H), 3.12 (d, 1H), 2.57-2.68 (m, 1H), 2.23-2.33 (m, 1H), 1.88-1.96 (m, 1H), 1.73-1.84 (m, 2H), 1.59-1.71 (m, 2H), 1.45-1.57 (m, 1H), 0.80-1.34 (m, 5H)

LC-MS: m/z 492 ($M+H^+$); m/z 514 ($M+Na^+$)

(iii) $MeOOCCH_2$-(*R*)Cgl-Aze-Pab-OOCPh

To a solution of H-(*R*)Cgl-Aze-Pab-OOCPh (480 mg; 0.97 mmol; from step (ii) above), K_2CO_3 (270 mg; 2 mmol) in acetonitrile (5 mL) at 20°C was added methyl bromoacetate (177 mg; 1.16 mmol). The reaction was stirred at 20°C for 14 h. The reaction mixture was filtered and concentrated to give a crude product which was purified by preparative RPLC to give gave 269 mg (49%) of the title compound as a white solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.43 (m, 1H, NH), 8.09 (m, 2H), 7.69 (m, 2H), 7.59 (m, 1H), 7.47 (m, 2H), 7.34 (m, 2H), 5.27 (s, 2H), 4.93 (dd, 1H), 4.59 (dd, 1H), 4.40 (dd, 1H), 4.12 (m, 2H), 3.65 (s, 3H), 2.87 (d, 1H), 2.72-2.63 (m, 1H), 2.55-2.48 (m, 1H), 1.96 (m, 1H), 1.74 (m, 2H), 1.67 (d, 1H), 1.59 (d, 1H), 1.56-1.50 (m, 1H), 1.29-1.08 (m, 4H), 1.04-0.94 (m, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) amidine and carbonyl signals: δ 175.1, 172.5, 170.6, 164.0, 157.1

LC-MS: m/z 564 ($M+H^+$)

Example 16

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH

To a solution of MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-OC(O)Ph (260 mg; 0.46 mmol; see Example 15(iii) above) in THF (4.6 mL) was added KOMe (1.6 mL; 0.29 M; 0.46 mmol) at 20°C. After 15 minutes of stirring the mixture was concentrated and subjected to preparative RPLC. This gave 109 mg (52%) of the title compound as a white solid.

^{1}H-NMR (500 MHz, MeOH-d4): δ 7.59 (d, 2H), 7.34 (d, 2H), 4.83 (s, 2H), 4.82-4.76 (m, 1H), 4.48 (d, 1H), 4.33 (d, 1H), 4.15-4.30 (m,2H), 3.64 (s, 3H), 3.04 (d, 1H), 2.57 (m, 1H), 2.26 (m, 1H), 1.95 (m, 1H), 1.75 (m, 2H), 1.58-1.70 (m, 2H), 1.53 (m, 1H), 1.31-1.10 (m, 4H), 1.04 (m, 1H)

^{13}C-NMR (100 MHz, MeOH-d4): amidine and carbonyl signals: δ 175.9, 174.3, 172.7, 155.2

LC-MS: m/z 460 ($M+H^+$), m/z 482 ($M+Na^+$)

Example 17

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH

To a solution of EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-C(O)OCH(CH$_3$)OOCCH$_3$ (184 mg; 0.31 mmol; see Example 14(iii) above), hydroxylamine hydrochloride (120mg; 1.72 mmol) and triethylamine (0.8 ml; 5.7 mmol) in EtOH (95%; 4.0 mL) was added, and the mixture stirred at room temperature for 4 days. The reaction mixture was concentrated and the crude product subjected to preparative RPLC. This gave 85mg (58%) of the title compound.

^{1}H-NMR (300 MHz, CD_3OD): δ 7.6 (d, 2H), 7.35 (d, 2H), 4.75-4.85 (m, 1H), 4.4-4.55 (m, 2H), 4.0-4.35 (m, 4H), 3.35 (d, 2H), 3.05 (d, 1H), 2.5-2.65 (m, 1H), 2.2-2.35 (m, 1H), 1.9-2.05 (m, 1H), 1.4-1.85 (m, 5H), 0.85-1.35 (m, 8H)

^{13}C-NMR (75.5 MHz, CD_3OD): amidine and carbonyl signals: δ 175.97, 173.91, 172.72, 155.23

LC-MS:(m+1)=474 (m/z)

Example 18

$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-OH

To a solution of hydroxylamine hydrochloride (320mg; 4.59mmol) and triethylamine (1.7ml; 12.24mmol) in EtOH, $BnOOCCH_2$-(*R*)Cgl-Aze-Pab-Z (1.0g; 1.52mmol) was added. The reaction mixture was stirred at room temperature for 40 hours and then concentrated. The crude product was purified by preparative RPLC using 50% acetonitrile in 0.1M NH_4OAc as eluent to give 0.34g (42%) of the title compound.

LC-MS:(m+1)=536 (m/z)

Example 19

n$PrOOCCH_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (700mg; 1.2mmol) and *n*-propyl bromoacetate (268mg; 1.45mmol). Yield 259mg (35%).

FAB-MS:(m+1)=606 (m/z)

Example 20

n$PrOOCCH_2$-(*R*)Cgl-Aze-Pab-OH

The title compound was prepared analogously to the procedure described in Example 18 from *n*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z (182mg; 0.3 mmol; see Example 19 above). The crude product was purified by preparative RPLC using 40% acetonitrile in 0.1M NH$_4$OAc as eluent to give 74mg (51%) of the desired compound.

LC-MS:(m+1)=488 (m/z)

Example 21

*i*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH

The title compound was prepared analogously to the procedure described in Example 18 from *i*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z (590mg; 0.7mmol; see Example 39 below). Yield 110 mg (32%)

LC-MS:(m+1)=488 (m/z)

Example 22

*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH

The title compound was prepared analogously to the procedure described in Example 18 from *t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z (738mg; 1.2mmol; see Example 37 below). Yield 290mg (48%).

LC-MS:(m+1)=502 (m/z)

Example 23

(*n*Pr)$_2$NCOCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab(OH)

(i) HOOCCH$_2$-(*R*)Cgl(Boc)-Aze-Pab-O-Boc

A solution of HOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH (670mg; 1.5mmol; see Example 28 below), (Boc)$_2$O (654mg; 3mmol), and DMAP (92mg; 0.75mmol) in THF:water (10:1) was stirred at room temperature for 2 h.

The reaction mixture was concentrated and purified by preparative RPLC. Freeze drying yielded 112mg (12%) of the sub-title compound as a white solid.

LC-MS:(m-1)=643 (m/z)

(ii) $(nPr)_2NCOCH_2OOCCH_2$-(*R*)Cgl(Boc)-Aze-Pab-O-Boc

A solution of $HOOCCH_2$-(*R*)Cgl(Boc)-Aze-Pab-O-Boc (100mg; 0.15mmol; from step (i) above), $(nPr)_2NCOCH_2OH$ (27mg; 0.17mmol; see Example 12(i) above), EDC (40mg; 0.21mmol) and DMAP (10mg; 0.075mmol) in acetonitrile (5mL) was stirred at room temperature for 4 days. The reaction mixture was concentrated, purified by preparative RPLC and freeze dried to give 21mg (18%) of the sub-title compound.

LC-MS:(m-1)=787 (m/z)

(iii) $(nPr)_2NCOCH_2OOCCH_2$-(*R*)Cgl-Aze-Pab-OH

A solution of $(nPr)_2NCOCH_2$-(*R*)Cgl(Boc)-Aze-Pab-O-Boc (20mg; 0.025mmol) in TFA:methylene chloride (1:1) was stirred at room temperature for 5 minutes. The reaction mixture was concentrated and freeze dried from acetonitrile and water to give 5mg (34%) of the title compound.

LC-MS:(m+1)=587 (m/z)

Example 24

$ChNHCOCH_2OOCCH_2$-(*R*)Cgl-Aze-Pab-OH

The title compound was prepared analogously to the procedure described in Example 18 from $ChNHCOCH_2OOCCH_2$-(*R*)Cgl-Aze-Pab-Z (118mg; 0.17mmol; see Example 11(iii) above). Yield 1.8 mg.

LC-MS:(m+1)=585 (m/z)

Example 25

MeNHCOCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-OH

The title compound was prepared analogously to the procedure described in Example 18 from MeNHCOCH$_2$-(*R*)Cgl-Aze-Pab-Z (81mg; 0.12mmol, see Example 36 below). Yield 10mg (16%).

LC-MS:(m+1)=517 (m/z)

Example 26

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OAc

(i) H-(*R*)Cgl-Aze-Pab-OAc

The sub-title compound was prepared analogously to the method described in Example 27 below (steps (i), (ii) and (iii)) using acetic acid anhydride instead of propanoic acid anhydride.

LC-MS:(m+1)=430 (m/z)

(ii) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OAc

The title compound was prepared analogously to the procedure described in Example 1(iii) above from H-(*R*)Cgl-Aze-Pab-OAc (370mg; 0.6mmol) and ethyl bromoacetate (105mg; 0.63mmol). Yield 67mg (22%).

LC-MS:(m+1)=516 (m/z)

Example 27

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OC(O)Et

(i) Boc-(*R*)Cgl-Aze-Pab-OH

To a solution of hydroxylamine hydrochloride and triethylamine in EtOH was added Boc-(*R*)Cgl-Aze-Pab-Z (1.0g; 1.52mmol). The reaction mixture was stirred at room temperature for 40 hours and then concentrated. The crude product was purified by preparative RPLC.

LC-MS: (m+1) = 488 m/z

(ii) Boc-(*R*)Cgl-Aze-Pab-OC(O)Et

A solution of Boc-(*R*)Cgl-Aze-Pab-OH (500mg; 0.91mmol; from step (i) above) and propanoic acid anhydride (3.5 mL) was stirred at room temperature for 45 minutes and then concentrated. The crude product was purified by preparative RPLC using 50% acetonitrile in 0.1M NH$_4$OAc as eluent to give 266mg (54%) of the sub-title compound.

LC-MS:(m+1)= 544(m/z)

(iii) H-(*R*)Cgl-Aze-Pab-OC(O)Et

The sub-title compound was prepared analogously to the procedure described in Example 1(ii) from Boc-(*R*)Cgl-Aze-Pab-OC(O)Et (238mg; 0.44mmol; from step (ii) above). Yield 290mg (100%).

LC-MS:(m+1)= 444(m/z)

(iv) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OC(O)Et

To a solution of H-(*R*)Cgl-Aze-Pab-OOCEt (300mg; 0.45mmol; from step (iii) above) and K$_2$CO$_3$ (308mg; 2.23mmol) in methylene chloride (6 mL) at 0°C was added dropwise $EtOOCCH_2OSO_2CF_3$ (105mg; 0.45mmol, prepared from triflic anhydride and ethyl glycolate). After the reaction mixture was stirred at room temperature for 1 h the reaction mixture was washed with water, citric acid and water, dried (Na_2SO_4) and concentrated. The crude product was purified by preparative RPLC using 45% acetonitrile in 0.1M NH_4OAc as eluent to give 63mg (27%) of the title compound.

LC-MS:(m+1)= 530(m/z)

Example 28

HOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH

(i) *t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-OOCPh

The sub-title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-OOCPh (250mg; 0.5mmol; see Example 15(ii) above) and *t*-butylbromoacetate (119mg; 0.6mmol). Yield 211mg (69%).

LC-MS:(m+1)=606 (m/z)

(ii) HOOCCH$_2$-(*R*)Cgl-Aze-Pab-OOCPh

The sub-title compound was prepared analogously to the procedure described in Example 1(ii) from *t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-OOCPh (233mg; 0.3mmol; from step (i) above). Yield 65mg (37%).

LC-MS:(m+1)=550 (m/z)

(iii) HOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH

A solution of HOOCCH$_2$-(*R*)Cgl-Aze-Pab-OOCPh (60mg; 0.1mmol; from step (ii) above) and KOMe (0.2M; 0.2mmol) in THF (10mL) and methanol (1.5mL) was stirred at room temperature for 5 minutes. The reaction mixture was concentrated and freeze dried from water and acetonitrile to give 28mg (63%) of the title compound.

LC-MS:(m+1)= 446(m/z)

Example 29

HOOCCH$_2$-(*R*)Cgl-Aze-Pab-O-*cis*-Oleyl

(i) tBuOOCCH$_2$-(*R*)Cgl(Boc)-Aze-Pab-Z

A solution of *t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z (1.7g, 2.8mmol; see Example 37 below), (Boc)$_2$O (672mg; 3.08mmol) and DMAP (68mg; 0.56mmol) in THF (30mL) was stirred at room temperature for 24 h. Additional (Boc)$_2$O (305mg; 1.4mmol) was added at 5°C. After another 24 h the reaction mixture was concentrated and purified by preparative RPLC to give 587mg (30%) of the desired compound.

EC-MS:(m+1)= 720 (m/z)

(ii) tBuOOCCH$_2$-(*R*)Cgl(Boc)-Aze-Pab-OH

The sub-title compound was prepared analogously to the procedure described in Example 18 from *t*BuOOCCH$_2$-(*R*)Cgl(Boc)-Aze-Pab-Z (580mg; 0.8mmol; from step (i) above). Yield 341mg (71%).

EC-MS:(m+1)= 602 (m/z)

(iii) tBuOOCCH$_2$-(*R*)Cgl(Boc)-Aze-Pab-O-*cis*-Oleyl

A solution of *t*BuOOCCH$_2$-(*R*)Cgl(Boc)-Aze-Pab-OH (340mg; 0.56mmol; from step (ii) above), *cis*-oleylchloride (170mg; 0.56mmol) and triethylamine (62mg; 0.61mmol) in methylene chloride was stirred for 5 minutes. The reaction mixture was concentrated and purified by preparative RPLC to give 326mg (67%) of the sub-title compound.

EC-MS:(m+1)= 867(m/z)

(iv) HOOCCH$_2$-(*R*)Cgl-Aze-Pab-O-*cis*-Oleyl

The title compound was prepared analogously to the procedure described in Example 1(ii) from *t*BuOOCCH$_2$-(*R*)Cgl(Boc)-Aze-Pab-O-*cis*-Oleyl (223mg; 0.25mmol; from step (iii) above).

LC-MS:(m+1)= 710 (m/z)

Example 30

Cyclooctyl-OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

(i) Cyclooctyl-bromoacetate

Cyclooctanol (1.3g; 10mmol) and DMAP (0.3g) was dissolved in methylene chloride followed by addition of bromacetyl chloride (1mL; 12mmol). After stirring for 18 h the reaction mixture was washed with aqueous Na$_2$CO$_3$ (2M) and HCl (1M), dried, concentrated and purified by flash chromatography using petroleum ether:methylene chloride (50:50) to give 1.8g (72%) of the sub-title compound.

(ii) Cyclooctyl-OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (703mg; 1.2mmol) and cyclooctyl bromoacetate (363mg; 1.46mmol; from step (i) above). Yield 379mg (46%).

FAB-MS:(m+1)= 674(m/z)

Example 31

tBuCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (2.5g; 4.3mmol) and tertbutylmetyl bromoacetate (1.08g; 5.2mmol). Yield 1.87g (69%).

FAB-MS:(m+1)= 634 (m/z)

Example 32

(2-Me)BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

(i) Methylbenzyl bromoacetate

The sub-title compound was prepared analogously to the procedure described in Example 30(i) from 2-methylbenzylalcohol (5g; 41mmol) and bromacetyl chloride (12.6g; 80mmol). Yield 8.2g (82%).

(ii) (2-Me)BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (580mg; 1mmol) and 2-methylbenzyl bromoacetate (290mg; 1.2mmol; from step (i) above). Yield 30mg (4.5%).

LC-MS:(m+1)= 668 (m/z)

Example 33

ChCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

A solution of BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z (1.41g; 1.7mmol) and cyclohexyl methylalcohol (6mL) in triethylamine (474 μL) and methylene chloride (3mL) was refluxed for 4 days. The reaction mixture was worked up to give a crude product which was purified by flash chromatography using methylene chloride:methanol (95:5) as eluent to give 801mg (71%) of the title compound.

FAB-MS:(m+1)= 660 (m/z)

Example 34

$ChOOCCH_2$-(*R*)Cgl-Aze-Pab-Z

(i) Cyclohexyl bromoacetate

The sub-title compound was prepared analogously to the procedure described in Example 32(i) above from cyclohexanol (1g; 10mmol) and bromacetylchloride (1mL; 12mmol).

(ii) $ChOOCCH_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (2.5g; 4.32mmol) and cyclohexyl bromoacetate (1.5g; 5.2mmol). Yield 1.7g (60%).

FAB-MS:(m+1)= 646 (m/z)

Example 35

$PhC(Me)_2OOCCH_2$-(*R*)Cgl-Aze-Pab-Z

(i) 2-Phenyl-2-propyl bromoacetate

The sub-title compound was prepared analogously to the procedure described in Example 30(i) from 2-phenyl-2-propanol (3g; 22mmol) and bromacetylchloride (4.16g, 26mmol). Yield 1.2g (44%).

(ii)$PhC(Me)_2OOCCH_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (1.2g; 2.2mmol) and 2-phenyl-2-propyl bromoacetate (640mg; 2.5mmol; from step (i) above). Yield 1.3g (86%).

^{1}H-NMR (500 MHz; $CDCl_3$) δ 9.3 (br s, 1H), 8.35 (t, 1H), 7.75 (d, 2H), 7.45 (d, 2H), 7.30-7.05 (m, 10H or 11H), 5.15 (s, 2H), 4.78 (t, 1H), 4.40-4.30 (AB part of ABX spectrum, 2H), 3.95 (q, 1H), 3.74 (q, 1H), 3.27-3.19 (AB-spectrum, 2H), 2.72 (d, 1H), 2.43 (q, 2H), 1.93 (br d, 1H), 1.75-1.60 (m, 9H or 10H), 1.54 (d, 1H), 1.49-1.40 (m, 1H), 1.25-1.0 (m, 4H), 0.92 (q, 1H)

Example 36

$MeNHCOCH_2OOCCH_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (1.0g; 1.7mmol) and $MeNHCOCH_2OOCCH_2Br$ (440mg; 2mmol; prepared analogously to the procedures described in Example 11 above (steps (i), (ii) and (iii)) using methylamine instead of cyclohexylamine). Yield 380mg (35%).

FAB-MS:(m+1)= 635 (m/z)

Example 37

t$BuOOCCH_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (500mg; 1.0mmol) and *t*-butyl bromoacetate (231mg; 1.2mmol). Yield 420mg (69%).

LC-MS:(m+1)= 620 (m/z)

Example 38

$(Me)_2CHC(Me)_2OOCCH_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z (787mg; 1.4mmol) and 2,3-dimethyl-2-butyl bromoacetate (364mg; 1.63mmol). Yield 590mg (67%).

FAB-MS:(m+1)= 648(m/z)

Example 39

i$PrOOCCH_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z (700mg; 1.2mmol) and isopropyl bromoacetate (262mg; 1.5mmol). Yield 225mg (31%)

FAB-MS:(m+1)= 606(m/z)

Example 40

$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe)

(i) Boc-(*R*)Cgl-Aze-Pab-COOPh(4-OMe)

The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(*R*)Cgl-Aze-Pab-H and 4-methoxyphenyl chloroformate.

FAB-MS:(m+1) = 622(m/z)

(ii) H-(*R*)Cgl-Aze-Pab-COOPh(4-OMe) x 2HCl

The sub-title compound was prepared analogously to the procedure described in Example 4(ii) from Boc-(*R*)Cgl-Aze-Pab-COOPh(4-OMe) (from step (i) above).

(iii) BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOPh(4-OMe) x 2HCl (85mg; 0.16mmol; from step (iii) above) and benzyl bromoacetate (90mg; 0.2mmol). Yield 60mg (56%).

FAB-MS:(m+1)= 670 (m/z)

Example 41

ChCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOPh(4-OMe) (554mg; 0.64mmol; see Example 40(ii) above) and cyclohexylmethyl bromoacetate (165mg; 0.7mmol). Yield 34mg (8%).

FAB-MS:(m+1)= 676 (m/z)

Example 42

(2-Me)BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOPh(4-OMe) (522mg; 1mmol; see Example 40(ii) above) and 2-(methyl)benzyl bromoacetate (365mg; 1.5mmol). Yield 158mg (23%).

LC-MS:(m+1)= 684 (m/z)

Example 43

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-Me)

(i) Boc-(*R*)Cgl-Aze-Pab-COOPh(4-Me)

The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(*R*)Cgl-Aze-Pab (1.96g; 4.56mmol) and 4-tolyl-chloroformate (850mg; 4.99mmol). Yield 1.39g (55%).

FAB-MS:(m+1)= 606(m/z)

(ii) H-(*R*)Cgl-Aze-Pab-COOPh(4-Me)

The sub-title compound was prepared analogously to the procedure described in Example 4(ii) from Boc-(*R*)Cgl-Aze-Pab-COOPh(4-Me) (388mg; 0.64mmol; from step (i) above). Yield 293mg (91%).

FAB-MS:(m+1)= 506(m/z)

(iii) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-Me)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOPh(4-Me) (288mg; 0.6mmol; from step (ii) above) and ethyl bromoacetate (114mg; 0.7mmol). Yield 81mg (24%).

FAB-MS:(m+1)= 592 (m/z)

Example 44

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-Me)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOPh(4-Me) (272mg; 0.54mmol; see Example 43(ii) above) and benzyl bromoacetate (147mg;

0.6mmol). Yield 107mg (31%).

FAB-MS:(m+1)= 654(m/z)

Example 45

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Bu

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-*n*Bu x 2HCl (400mg; 0.74mmol; see Example 9(ii) above) and benzyl bromoacetate (210mg; 0.88mmol). Yield 220mg (48%).

FAB-MS:(m+1)= 620 (m/z)

Example 46

*i*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$ x 2TFA (456mg; 0.67mmol; see Example 1(ii) above) and isopropyl bromoacetate (145mg; 0.8mmol). Yield 294mg (79%).

FAB-MS:(m+1)= 556 (m/z)

Example 47

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*i*Bu

(i) Boc-Pab-COO-*i*Bu

To a solution of Boc-Pab-H (500mg; 2.0 mmol; prepared from Pab-Z and (Boc)$_2$O (forming Boc-Pab-Z), followed by hydrogenation over Pd/C) and triethylamine (400mg; 4.0mmol) in methylene chloride (10mL) was added *i*-butyl chloroformate (270mg; 2.2mmol) at 0°C. After stirring for 5 h, water was added. The organic phase was dried (Na_2SO_4) and concentrated to give 530mg (76%) of the sub-title compound.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.5 (bs, 1H), 7.82 (d, 2H), 7.31 (d, 2H), 6.6 (bs, 1H), 5.0 (bs, 1H), 4.33 (bd, 2H), 3.93 (d, 2H), 2.04 (m, 1H), 1.45 (s, 9H), 0.97 (d, 6H)

(ii) H-Pab-COO-*i*Bu x 2HCl

The sub-title compound was prepared analogously to the procedure described in Example 4(ii) from Boc-Pab-COO-*i*Bu (520mg; 1.5mmol; from step (i) above). Yield 430mg (88%).

^{1}H-NMR (500 MHz, MeOD) δ 7.89 (d, 2H), 7.75 (d, 2H), 4.30 (s, 2H), 4.17 (d, 2H), 2.11-2.05 (m, 1H), 1.02 (d, 6H)

(iii) Boc-(*R*)Cgl-Aze-Pab-COO-*i*Bu

To a solution of Boc-(*R*)Cgl-Aze-OH (480mg; 1.4mmol), H-Pab-COO-*i*Bu x 2HCl (430mg; 1.3mmol; from step (ii) above) and DMAP (650mg; 5.3mmol) in acetonitrile (20mL) was added EDC (270mg; 1.4mmol). After stirring for 3 days at room temperature the reaction mixture was concentrated and then dissolved in water and EtOAc. The organic phase was washed with $NaHCO_3$ (aq) and dried (Na_2SO_4), concentrated and purified by flash chromatography using EtOAc as eluent to give 510mg (52%) of the sub-title compound.

(iv) H-(*R*)Cgl-Aze-Pab-COO-*i*Bu x 2HCl

The sub-title compound was prepared analogously to the procedure described in Example 4(ii) from Boc-(*R*)Cgl-Aze-Pab-COO-*i*Bu (500mg; 0.88mmol; from step (iii) above). Yield 360mg (87%).

(v) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*i*Bu

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-*i*Bu x 2HCl (290mg; 0.53mmol; from step (iv) above) and ethyl bromoacetate (110mg; 0.64mmol). Yield 140mg (47%).

FAB-MS:(m+1)= 558 (m/z)

Example 48

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Pr

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-*n*Pr x 2TFA (902mg; 1.3mmol; see Example 5(ii) above) and benzyl bromoacetate (362mg; 1.6 mmol). Yield 199mg (25%).

^{1}H-NMR: (400MHz; CDCl$_3$) δ 8.43 (bs, 1H), 7.78 (d, 2H), 7.38-7.27 (m, 7H), 5.05 (s, 2H), 4.90 (dd, 1H), 4.56-4.39 (AB part of ABX spectrum, 2H), 4.12-4.03 (m, 3H), 3.98-3.91 (q, 1H), 3.33-3.22 (AB-spectrum, 2H), 2.85 (d, 1H), 2.65-0.94 (m, 19H)

Example 49

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCCh

(i) EtSCOOCH$_2$OOCCh

To a solution of tetrabutylammonium hydrogensulphate (15.6g, 45.6mmol) and cyclohexane carboxylic acid (5.85g, 46mmol) in methylene chloride was added NaOH (9.1mL,10M; 68mmol) at 0°C. After stirring for 5 minutes the reaction mixture was filtered, washed with methylene chloride, dissolved in toluene, concentrated and dissolved in THF to give [Bu$_4$N]$^+$[OOCCh]$^-$. EtSCOOCH$_2$Cl (4g; 25.9mmol; see Folkmann and

Lund, J. Synthesis, (1990), 1159) was added to the THF solution of $[Bu_4N]^+[OOCCh]^-$ at room temperature. After stirring at room temperature for 12 h the reaction mixture was concentrated and purified by flash chromatography to give 2.57g (40%) of the sub-title compound.

^{1}H-NMR (400 MHz, $CDCl_3$) diagnostic peaks δ 5.80 (s, 2H, O-CH_2-O), 2.85 (q, 2H, CH_2-S)

(ii) $ClCOOCH_2OOCCh$

To $EtSCOOCH_2OOCCh$ (2.9g; 11.8mmol; from step (i) above) was added dropwise SO_2Cl_2 (3.18g; 23.6mmol) at 0°C. After stirring for 30 minutes the reaction mixture was concentrated to give 1.82g (70%) of the desired compound.

^{1}H-NMR (500 MHz, $CDCl_3$) diagnostic peaks δ 5.82 (s, 2H, O-CH_2-O)

(iii) Boc-(*R*)Cgl-Aze-Pab-$COOCH_2OOCCh$

The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(*R*)Cgl-Aze-Pab-H (750mg; 1.59mmol) and $ClCOOCH_2OOCCh$ (460mg; 2.1mmol; from step (ii) above). The crude product was purified by preparative RPLC. Yield 355mg (9%).

FAB-MS:(m+1)= 656(m/z)

(iv) H-(*R*)Cgl-Aze-Pab-$COOCH_2OOCCh$ x 2TFA

The sub-title compound was prepared analogously to the procedure described in Example 1(ii) from Boc-(*R*)Cgl-Aze-Pab-$COOCH_2OOCCh$ (from step (iii) above).

(v) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCCh

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCCh x 2TFA (193mg; 0.35mmol; from step (iv) above) and ethyl trifluoroacetate (83mg; 0.35mmol). Yield 87mg (39%).

^{1}H-NMR (400 MHz, CDCl$_3$) δ 8.48 (t br, 1H), 7.83 (d, 2H), 7.37 (d, 2H), 5.86 (s, 2H), 4.95 (dd, 1H), 4.15-4.39 (AB part of ABX spectrum, 2H), 4.18-4.05 (m, 5H), 3.26-3.17 (AB-spectrum, 2H), 2.87 (d, 1H), 2.75-0.95 (m, 29H)

Example 50

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCCH$_2$Ch

The title compound was prepared analogously to the procedure described in Example 49 above starting with cyclohexyl acetic acid instead of cyclohexane carboxylic acid. Yield 74mg (17%).

FAB-MS:(m+1)= 656(m/z)

Example 51

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH(Me)OOCPh

The title compound was prepared analogously to the procedure described in Example 49 above starting with EtSCOOCH(CH$_3$)Cl (prepared from ClCOCH(CH$_3$)Cl and EtSH using the procedure described by Folkmann *et al* in J. Synthesis, (1990), 1159) instead of EtSCOOCH$_2$Cl. Yield 70mg (23%).

FAB-MS:(m+1)= 650 (m/z)

Example 52

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCPh

The title compound was prepared analogously to the procedure described in Example 49 above using benzoic acid instead of cyclohexane carboxylic acid. Yield 50mg (39%).

^{1}H-NMR (300 MHz, CDCl$_3$) δ 9.73-9.25 (s br, 1H), 8.45 (t, 1H), 8.05 (d, 2H), 7.83 (d, 2H), 7.60-7.10 (m, 6H), 6.10 (s, 2H), 4.96-4.84 (dd, 1H), 4.62-4.30 (ABX, 2H), 4.20-3.93 (m, 4H), 3.25 (s, 2H), 2.84 (d, 1H), 2.73-2.41 (m, 2H), 2.41-0.87 (m, 15H)
^{13}C-NMR (300 MHz, CDCl$_3$, amidine and carbonyl carbons) δ 163.1, 165.3, 169.0, 170.8, 172.3, 175.5

Example 53

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH(Me)OAc

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH(CH$_3$)OC(O)CH$_3$ (108mg; 0.21mmol; see Example 14(ii) above) and benzyl bromoacetate (36μL; 0.23mmol). Yield 41mg (30%).

FAB-MS:(m+1)= 650(m/z)

Example 54

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OAc

(i) H-(*R*)Cgl-Aze-Pab-COOCH$_2$OAc x 2TFA

The sub-title compound was prepared analogously to the procedure described in Example 14 (steps (i) and (ii)) above using acetoxymethyl 4-nitrophenylcarbonate (prepared analogously to the method described in Example 12(iii) using silver acetate instead of silver pivalate). Work up gave the sub-title compound which was used in the next step without further purification.

(ii) EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OAc

The title compound was prepared analogously to the procedure described in Example 1(iii) above from H-(*R*)Cgl-Aze-Pab-COOCH$_2$OAc x 2TFA (0.83mmol; from step (i) above) and ethyl bromoacetate (2.2mmol). Yield 286mg.

FAB-MS:(m+1)= 574 (m/z)

Example 55

*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OAc

The title compound was prepared analogously to the procedure described in Example 1(iii) above from H-(*R*)Cgl-Aze-Pab-COOCH$_2$OAc x 2TFA (0.313mmol; see Example 54(i) above) and *t*-butyl bromoacetate (73mg; 0.376mmol). Yield 156mg (83%).

FAB-MS:(m+1)= 602(m/z)

Example 56

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOC-*t*Bu

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH$_2$OOC-*t*Bu (379mg; 0.71mmol; see Example 12(v) above) and benzyl bromoacetate (135μL; 0.85mmol). Yield 146mg (30%).

FAB-MS:(m+1)= 678(m/z)

Example 57

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CCl$_3$

(i) Boc-(*R*)Cgl-Aze-Pab-COOCH$_2$CCl$_3$

The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(*R*)Cgl-Aze-Pab-H (1.0g; 2.12 mmol), 2M NaOH (11.7 ml) and trichloroethyl chloroformate (494 mg; 2.33 mmol). Yield 1.08g (79%).

(ii) H-(*R*)Cgl-Aze-Pab-COOCH$_2$CCl$_3$

The sub-title compound was prepared analougously to the procedure described in Example 1(ii) from Boc-(*R*)Cgl-Aze-Pab-COOCH$_2$CCl$_3$ (1.04g; 1.607 mmol; from step (i) above). Yield 1.43g (99%).

^{1}H-NMR: (500MHz; CD$_3$OD) δ 7.79 (d, 2H), 7.61 (d, 2H), 5.10 (s, 2H), 4.87-4.81 (m, 2H), 4.63-4.52 (q, 2H), 4.41-4.34 (m, 1H), 4.30-4.24 (m, 1H), 3.72 (d, 1H), 2.72-2.63 (m, 1H), 2.32-2.25 (m, 1H), 1.88-1.10 (m, 14H)

(iii) EtOOCCH$_2$-(*R*)Cgl-Aze Pab-COOCH$_2$CCl$_3$

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH$_2$CCl$_3$ (400 mg; 0.52 mmol; from step (ii) above) and ethyl bromoacetate (95 mg; 0.57 mmol). Yield 8 mg (23%).

^{1}H-NMR: (500MHz; CDCl$_3$) δ 8.47 (bt, 1H), 7.83 (d, 2H), 7.48 (bs, 1H), 7.31 (d, 2H), 4.92 (dd, 1H), 4.85 (s, 2H), 4.58-4.39 (AB part of ABX spectrum, 2H), 4.16-4.06 (m, 4H), 3.24 (s, 2H), 4.87 (d, 1H), 2.65-2.59 (m, 1H), 2.56-2.48 (m, 1H), 2.10-0.95 (m, 16H)

Example 58

MeOOC-C(=CEt)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

(i) MeOOC-C(=CH)C(OH)Et

Propionaldehyde (10.1 g; 0.174 mol) was added dropwise to a solution of methyl acrylate (10g; 0.116mol) and 1,4-diazobicyclo[2,2,2]octane (1.3 g; 0.0116 mol). The reaction mixture was stirred at room temperature for 14 days. Ethyl acetate (150 ml) was added. The organic phase was washed with water and brine, dried (Na$_2$SO$_4$), filtered and concentrated to give the desired compound. Yield 15.5g (93%).

^{1}H-NMR: (400MHz; CDCl$_3$) δ 6.24 (s, 1H), 5.81 (s, 1H), 4.34 (t, 1H), 3.78 (s, 3H), 2.82 (bs, 1H), 1.69 (m, 2H), 0.95 (t, 3H)

(ii) MeOOC-C(=CEt)CH$_2$Br

HBr (6.5 ml, ~48%) was added dropwise to MeOOC-C(=CH)C(OH)Et (3 g; 20.8 mmol; from step (i) above) at 0°C. After 5 minutes H$_2$SO$_4$ (conc.; 6 ml) was added dropwise. The reaction mixture was stirred for 12 hours at room temperature. Two phases was separated and the top phase was diluted with ether. The ether phase was washed with water and aqueous NaHCO$_3$, dried (Na$_2$SO$_4$ and charcoal) and concentrated. The residue was purified by flash chromatography. Yield 1.7g (40%).

^{1}H-NMR: (400MHz; CDCl$_3$) δ 6.97 (t, 3H), 4.23 (s, 2H), 3.8 (s, 3H), 2.32 (m, 2H), 1.13 (t, 3H)

(iii) *t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

The sub-title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z (2.1 g; 3.6 mmol) and *t*-butyl bromoacetate (780 mg; 4.0 mmol). Yield 1.73g (78%).

(iv) HOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

A solution of *t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z (from step (iii) above) and TFA in methylene chloride was stirred in room temperature for 3 h. The rection mixture was concentrated and freeze dried from water and HCl (conc.; 10 eq.).

(v) MeOOC-C(=CEt)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

A solution of HOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z (263 mg; 0.41 mmol; from step (iv) above), NaOH (1M; 1.239 ml; 1.239 mmol) and water (4 ml) was freeze dried. DMF (5 ml) was added, followed by dropwise addition of Me-OOC-C(=CEt)CH$_2$Br (103 mg; 0.496 mmol; from step (ii) above) at 0°C. The reaction mixture was stirred for 24 h at room temperature, diluted with toluene (5 ml), washed with water, dried (Na$_2$SO$_4$) and concentrated. The residue was purified by flash chromatography using EtOAc:methanol (95:5) as eluent. Yield 95 mg (33%).

FAB-MS:(m+1)= 690 (m/z)

Example 59

MenOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe)

(i) MenOOCCH$_2$Br

The sub-title compound was prepared analogously to the procedure described in Example 30(i) above from MenOH (10 mmol) and bromoacetyl chloride (12 mmol). Yield 1.5g (54%).

(ii) MenOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-Ph(4-OMe) (521 mg; 1 mmol; see Example 40(ii) above) and MenOOCCH$_2$Br (416 mg; 1.5

mmol; from step (i) above). Yield 36 mg (5%).

FAB-MS:(m+1)= 718 (m/z)

Example 60

*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO*n*Pr

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-*n*Pr (575 mg; 0.837 mmol; see Example 5(ii) above) and *t*-butyl bromoacetate (196 mg; 1.01 mmol). Yield 110 mg (23%).

LC-MS:(m+1)=572 (m/z)

Example 61

MenOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z (0.7 g; 1.21 mmol) and MenOOCCH$_2$Br (0.4 g; 1.45 mmol; see Example 59(i) above). Yield 0.33g (38%).

FAB-MS:(m+1)= 702 (m/z)

Example 62

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO$_2$)

(i) Boc-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO$_2$)

The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(*R*)Cgl-Aze-Pab-H (1.03g; 2.18 mmol), 2M NaOH (24 mL) and 4-NO$_2$-benzyl chloroformate (518 mg; 2.4 mmol). Yield 1.32g (93%).

FAB-MS:(m+1)=651(m/z)

(ii) H-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO_2)

The sub-title compound was prepared analogously to the procedure described in Example 4(ii) from Boc-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO_2) (1.32 mg; 2.03 mmol; from step (i) above). Yield 1.0g (79%).

FAB-MS:(m+1)=551(m/z)

(iii) BnOOCCH_2-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO_2)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO_2) (0.5g; 0.80mmol; from step (ii) above) and benzyl bromoacetate (220 mg; 0.90 mmol).

FAB-MS:(m+1)=699(m/z)

Example 63

EtOOCCH_2-(*R*)Cgl-Aze-Pab-Bn(4-NO_2)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO_2) (211 mg; 0.38mmol; see Example 62(ii) above) and ethyl bromoacetate (47 μl; 0.42 mmol). Yield 44 mg (18%).

^{1}H-NMR: (300MHz; CDCl3) δ 9.55 (bs, 1H), 8.50 (bt, 1H), 8.20 (d, 2H), 7.80 (d, 2H), 7.60 (d, 2H), 7.35 (d, 2H), 6.87 (bs, 1H), 4.95 (dd, 1H), 4.65-4.40 (AB part of ABX spectrum, 2H), 4.18-4.04 (m, 5H), 3.27-3.15 (AB-spectrum, 2H), 2.87 (d, 1H), 2.75-2.60 (m, 1H), 2.57-2.45 (m, 1H), 2.00-0.95 (m, 16H).

Example 64

PrlC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

(i) PrlC(O)CH$_2$OH

A mixture of 2,5-dioxo-1,4-dioxane (2.0 g; 17 mmol) and pyrrolidine (8 ml; 97 mmol) was refluxed for 1 h. The excess pyrrolidine was removed by evaporation. Yield 4.4g (99%).

FAB-MS(m+1)=130(m/z)

(ii) PrlC(O)CH$_2$OOCCH$_2$Br

To a solution of PrlC(O)CH$_2$OH (0.4 g; 3.1 mmol; from step (i) above) in DMF (15 ml) was added dropwise bromoacetyl bromide (0.63 g; 3.1 mmol) at 0°C. The reaction mixture was stirred for 1.5 h at 0°C and 3 h at room temperature. Additional bromoacetyl bromide (0.63 g; 3.1 mmol) was added and the reaction mixture was heated to 80°C, stirred at room temperature for 12 h and concentrated. Yield 320 mg (41%)

FAB-MS(m+1)=252(m/z)

(iii) PrlC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z (580 mg; 1 mmol) and PrlC(O)CH$_2$OOCCH$_2$Br (300 mg; 1.2 mmol; from step (ii) above). Yield 400 mg (60%).

FAB-MS(m+1)=675 (m/z)

^{1}H-NMR: (500MHz; CDCl3) δ 9.66-9.42 (bs, 1H), 8.64-8.56 (m, 1H), 8.03-7.93 (d, 2H), 7.89-7.66 (bs, 1H), 7.45 (d, 2H), 7.45-7.25 (m, 5H), 5.20 (s, 2H), 4.98-4.92 (dd, 1H), 4.82-4.74 (m, 1H), 4.62, 4.58 (AB spectrum, 2H), 4.26-4.05 (m, 3H),3.47-3.16 (m, 6H), 2.95 (d,1H), 2.78-2.68 (m, 1H), 2.54-2.42 (m, 1H), 2.03-1.95 (m, 16H)

Example 65

(2-Me)BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO$_2$)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO$_2$) (500 mg; 0.80mmol; see Example 62(ii) above) and 2-(methyl)benzyl bromoacetate (234 mg; 0.96 mmol; see Example 32(i) above). Yield 528 mg (92%).

^{1}H-NMR: (400MHz, CDCl$_3$) δ 9.34 (bs, 1H), 8.38 (t, 1H), 8.09 (d, 2H), 7.72 (d, 2H), 7.48 (d, 2H), 7.37 (bs, 1H), 7.23 (d, 2H), 7.17-7.05 (m, 4H), 5.18 (s, 2H), 5.00 (s, 2H), 4.81 (dd, 1H), 4.45-4.34 (AB part of ABX spectrum, 2H), 4.04-3.97 (q, 1H), 3.93-3.86 (q, 1H), 3.27-3.17 (AB spectrum, 2H), 2.79 (d, 1H), 2.54-2.35 (m,2H), 2.22 (s, 3H), 1.91-1.84 (bd, 1H),1.71-1.39 (m, 5H), 1.19-0.84 (m, 4H).

Example 66

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOEt

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOEt (305 mg; 0.69 mmol; see Example 4(ii) and methyl bromoacetate (126 mg; 0.83 mmol). Yield 188 mg (53%).

LC-MS:(m+1)=516(m/z)

Example 67

(*n*Pr)$_2$NC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO$_2$)

(i) (*n*Pr)$_2$NC(O)CH$_2$OOCCH$_2$Cl

A mixture of (*n*Pr)$_2$NC(O)CH$_2$OH (244 mg; 1.53 mmol; see Example 12(i) above) and bromacetyl chloride (270 mg; 1.72 mmol) was stirred at room temperature for 12 hours. The mixture was poured into aqueous NaHCO$_3$ and extracted with methylene chloride. The organic phase was washed with aqueous KHSO$_4$ (0.2M) and brine, dried and concentrated.

FAB-MS:(m+1)=237(m/z)

^{1}H-NMR: (400MHz, CDCl$_3$) δ 4.82 (s, 2H), 4.22 (s, 2H), 3.31-3.26 (t, 2H), 3.10-3.15 (t, 2H), 1.68-1.52 (m, 2H), 1.97-0.86 (m, 6H)

(ii) (*n*Pr)$_2$NC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-Bn(4-NO$_2$)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOBn(4-NO$_2$) (343 mg; 0.62mmol; see Example 62(ii) above) and (*n*Pr)$_2$NC(O)CH$_2$OOCCH$_2$Cl (160 mg; 0.68 mmol; from step (i) above). Yield 89 mg (19%).

FAB-MS:(m+1)=750(m/z)

Example 68

(2-Me)BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOC*t*Bu

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH$_2$OOC*t*Bu (380 mg; 0.71 mmol; see Example 12(v) above) and 2-(methyl)benzyl bromoacetate (215 mg; 0.88 mmol; see Example 32(i) above). Yield 37 mg (7.5%).

FAB-MS:(m+1)=692(m/z)

Example 69

The compounds of Examples 1 to 68 were all tested in Test A above and were all found to exhibit an $IC_{50}TT$ value of more than 1.0μM (ie they were are inactive to thrombin *per se*; cf. the active inhibitor HOOC-CH_2-(*R*)Cgl-Aze-Pab-H which exhibits an $IC_{50}TT$ of 0.01 μM).

Example 70

The compounds of Examples 1 to 68 were tested in one, two or all of Tests B, C and/or D above, and were all found to exhibit oral and/or parenteral bioavailability in the rat as the active inhibitor HOOC-CH_2-(*R*)Cgl-Aze-Pab-H, either as the free acid and/or as one or more ester thereof. Based on the assumption that HOOC-CH_2-(*R*)Cgl-Aze-Pab-H is formed in the rat, the bioavailability was calculated according to the formulae described in Test B and/or Test C as appropriate.

Abbreviations

Ac = acetyl
aq = aqueous
Aze = S-Azetidine-2-carboxylic acid
Boc = *t*-butyloxycarbonyl
$(Boc)_2O$ = di-*t*-butyldicarbonate
Bn = benzyl
Bu = butyl
Cgl = cyclohexylglycine
Ch = cyclohexyl
DCC = dicyclohexyl carbodiimide
DMAP = N,N-dimethyl amino pyridine
EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

Et = ethyl

EtOH = ethanol

EtOAc= ethyl acetate

h = hours

HCl = hydrochloric acid

H-Pab-H = 1-amidino-4-aminomethyl benzene

H-Pab-Z = 4-aminomethyl-1-(N-benzyloxycarbonylamidino) benzene

HPLC = high performance liquid chromatography

K_2CO_3= anhydrous potassium carbonate

Me = methyl

Men = (1*R*,2*S*,5*R*)-menthyl

Pab-OH= 4-aminomethyl-benzamidoxime (4-aminomethyl-1-(amino-hydroxyiminomethyl) benzene

Piv(aloyl) = 2,2-dimethylacetyl

Pr = propyl

Prl = N-pyrrolidinyl

RPLC = reverse phase high performace liquid chromatography

TFA = trifluoroacetic acid

THF = tetrahydrofuran

Z = benzyloxy carbonyl

Prefixes *n*, *s*, *i* and *t* have their usual meanings: normal, iso, sec and tertiary. Prefixes in the NMR-spectra s, d, t, q, and b mean singlet, doublet, triplet, quartet, and broad, respectively. The stereochemistry for the amino acids is by default (S) if not otherwise stated.

Claims

1. A compound of formula I,

$$R^1O(O)C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}R^2 \qquad \text{I}$$

wherein

R^1 represents $-R^3$ or $-A^1C(O)N(R^4)R^5$ or $-A^1C(O)OR^4$;

A^1 represents C_{1-5} alkylene;

R^2 (which replaces one of the hydrogen atoms in the amidino unit of Pab-H) represents OH, $OC(O)R^6$, $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$;

R^3 represents H, C_{1-10} alkyl, or C_{1-3} alkylphenyl (which latter group is optionally substituted by C_{1-6} alkyl, C_{1-6} alkoxy, nitro or halogen);

R^4 and R^5 independently represent H, C_{1-6} alkyl, phenyl, 2-naphthyl or, when R^1 represents $-A^1C(O)N(R^4)R^5$, together with the nitrogen atom to which they are attached represent pyrrolidinyl or piperidinyl;

R^6 represents C_{1-17} alkyl, phenyl or 2-naphthyl (all of which are optionally substituted by C_{1-6} alkyl or halogen);

R^7 represents 2-naphthyl, phenyl, C_{1-3} alkylphenyl (which latter three groups are optionally substituted by C_{1-6} alkyl, C_{1-6} alkoxy, nitro or halogen), or C_{1-12} alkyl (which latter group is optionally substituted by C_{1-6} alkoxy, C_{1-6} acyloxy or halogen);

R^8 represents H or C_{1-4} alkyl; and

R^9 represents 2-naphthyl, phenyl, C_{1-6} alkoxy or C_{1-8} alkyl (which latter group is optionally substituted by halogen, C_{1-6} alkoxy or C_{1-6} acyloxy);

provided that when R^1 represents R^3, R^3 represents benzyl, methyl, ethyl, *n*-butyl or *n*-hexyl and R^2 represents $C(O)OR^7$, then R^7 does not represent benzyl;

or a pharmaceutically-acceptable salt thereof.

2. A compound of formula I, as defined in Claim 1, wherein A^1 represents C_{1-3} alkylene when R^1 represents $-A^1C(O)N(R^4)R^5$.

3. A compound of formula I, as defined in Claim 1 or Claim 2, wherein R^4 represents H or C_{1-6} alkyl when R^1 represents $-A^1C(O)N(R^4)R^5$.

4. A compound of formula I, as defined in any one of Claims 1 to 3, wherein R^5 represents C_{1-6} alkyl or C_{4-6} cycloalkyl when R^1 represents $-A^1C(O)N(R^4)R^5$.

5. A compound of formula I, as defined in any one of Claims 1 to 3, wherein R^4 and R^5 together represent pyrrolidinyl when R^1 represents $-A^1C(O)N(R^4)R^5$.

6. A compound of formula I, as defined in any one of Claims 2 to 5, wherein A^1 represents C_{1-3} alkylene, and R^4 represents H or C_{1-3} alkyl and R^5 represents C_{2-6} alkyl or C_{5-6} cycloalkyl, or R^4 and R^5 together represent pyrrolidinyl.

7. A compound of formula I, as defined in Claim 1, wherein A^1 represents C_{1-5} alkylene when R^1 represents $-A^1C(O)OR^4$.

8. A compound of formula I, as defined in Claim 1 or Claim 7, wherein R^4 represents C_{1-6} alkyl when R^1 represents $-A^1C(O)OR^4$.

9. A compound of formula I, as defined in Claim 7 or Claim 8, wherein A^1 represents C_{1-5} alkylene and R^4 represents C_{1-4} alkyl.

10. A compound of formula I, as defined in Claim 1, wherein R^3 represents H, C_{1-10} alkyl (which latter group may be linear or, when there are a sufficient number of carbon atoms, may be branched and/or be partially cyclic or cyclic), or C_{1-3} alkylphenyl (which latter groups is optionally substituted, may be linear or, when there are a sufficient

number of carbon atoms, be branched), when R^1 represents R^3.

11. A compound as claimed in Claim 1 or Claim 10, wherein R^1 represents H, linear C_{1-10} alkyl, branched C_{3-10} alkyl, partially cyclic C_{4-10} alkyl, C_{4-10} cycloalkyl, optionally substituted linear C_{1-3} alkylphenyl, optionally substituted branched C_3 alkylphenyl.

12. A compound as claimed in Claim 11, wherein R^1 represents linear C_{1-6} alkyl, C_{6-10} cycloalkyl, or optionally substituted linear C_{1-3} alkylphenyl.

13. A compound of formula I, as defined in any one of Claims 1 to 12, wherein R^2 represents OH.

14. A compound of formula I, as defined in any one of Claims 1 to 12, wherein R^6 represents optionally substituted phenyl or C_{1-17} alkyl (which latter group may be linear or, when there are a sufficient number of carbon atoms, may be branched, be cyclic or partially cyclic, and/or be saturated or unsaturated) when R^2 represents $OC(O)R^6$.

15. A compound as claimed in Claim 14 wherein R^6 represents optionally substituted phenyl, linear C_{1-4} alkyl, branched C_{3-4} alkyl or *cis*-oleyl.

16. A compound as claimed in Claim 15 wherein R^6 represents linear C_{1-3} alkyl or branched C_3 alkyl.

17. A compound of formula I, as defined in any one of Claims 1 to 12, wherein R^7 represents optionally substituted phenyl, C_{1-12} alkyl (which latter group is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, may be branched, cyclic or partially cyclic, and/or saturated or unsaturated), or C_{1-3} alkylphenyl (which latter

group is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, may be branched) when R^2 represents $C(O)OR^7$.

18. A compound as claimed in Claim 17 wherein R^7 represents optionally substituted and/or optionally unsaturated linear C_{1-4} alkyl or optionally substituted and/or optionally unsaturated branched C_{3-4} alkyl, optionally substituted phenyl, or optionally substituted linear C_{1-3} alkylphenyl or optionally substituted branched C_3 alkylphenyl.

19. A compound as claimed in Claim 18 wherein R^7 represents optionally substituted linear C_{1-4} alkyl or optionally substituted branched C_{3-4} alkyl, optionally substituted linear C_{1-3} alkylphenyl or branched C_3 alkylphenyl.

20. A compound of formula I, as defined in any one of Claims 1 to 12, wherein R^8 represents H or methyl, when R^2 represents $C(O)OCH(R^8)OC(O)R^9$.

21. A compound of formula I, as defined in any one of Claims 1 to 12 or Claim 20, wherein R^9 represents phenyl, or C_{1-8} alkyl (which latter group is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, may be branched and/or cyclic or partially cyclic) when R^2 represents $C(O)OCH(R^8)OC(O)R^9$.

22. A compound of formula I, as defined in Claim 20 or Claim 21 wherein R^8 represents H or methyl and R^9 represents phenyl, C_{5-7} cycloalkyl, linear C_{1-6} alkyl, branched C_{3-6} alkyl or partially cyclic C_{7-8} alkyl.

23. A compound as claimed in Claim 22 wherein R^8 represents H and R^9

represents C_{5-7} cycloalkyl, linear C_{1-6} alkyl or partially cyclic C_{7-8} alkyl.

24. A compound as claimed in any one of the preceeding claims wherein, when R^1 represents R^3 and R^3 represents optionally substituted C_{1-3} alkylphenyl, the optional substituent C_{1-6} alkyl.

25. A compound as claimed in Claim 24 wherein the substituent is methyl.

26. A compound as claimed in any one of the preceeding claims wherein, when R^2 represents $C(O)OR^7$ and R^7 represents optionally subsituted C_{1-12} alkyl, the optional substituent is selected from halogen and C_{1-6} alkoxy.

27. A compound as claimed in Claim 26 wherein the substituent is selected from chloro and methoxy.

28. A compound as claimed in any one of the preceeding claims wherein, when R^2 represents $C(O)OR^7$ and R^7 represents optionally subsituted phenyl, the optional substituent is selected from C_{1-6} alkyl, C_{1-6} alkoxy and halogen.

29. A compound as claimed in Claim 28 wherein the substituent is selected from methyl, methoxy and chloro.

30. A compound as claimed in any one of the preceeding claims wherein when R^2 represents $C(O)OR^7$ and R^7 represents optionally subsituted C_{1-3} alkylphenyl, the optional substituent is nitro.

31. A compound as claimed in Claim 1 which is

$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2CH{=}CH_2$;

n$PrOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2CH{=}CH_2$;

*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$CH=CH$_2$;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOEt;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COO-*n*Bu;

PrlC(O)CH$_2$CH$_2$CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

ChNHC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

(*n*Pr)$_2$NC(O)CH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCC(CH$_3$)$_3$;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH$_2$OOCC(CH$_3$)$_3$;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOCH(CH$_3$)OOCCH$_3$;

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-OOCPh;

MeOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

*n*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

*n*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

*i*PrOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

*t*BuOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

(*n*Pr)$_2$NCOCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

ChNHCOCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

EtOOCCH$_2$-(*R*)Cgl-Aze-Pab-OAc;

HOOCCH$_2$-(*R*)Cgl-Aze-Pab-OH;

HOOCCH$_2$-(*R*)Cgl-Aze-Pab-O-*cis*-Oleyl;

Cyclooctyl-OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

*t*BuCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

(2-Me)BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

ChCH$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

ChOOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

PhC(Me)$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

(Me)$_2$CHC(Me)$_2$OOCCH$_2$-(*R*)Cgl-Aze-Pab-Z;

BnOOCCH$_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe);

$ChCH_2OOCCH_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe);
(2-Me)$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe);
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-COOPh(4-Me);
$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-COOPh(4-Me);
$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-COO-*n*Bu;
i$PrOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2CH=CH_2$;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-COO-*i*Bu;
$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-COO-*n*Pr;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2OOCCh$;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2OOCCH_2Ch$;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-COOCH(Me)OOCPh;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2OOCPh$;
$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-COOCH(Me)OAc;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2OAc$;
t$BuOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2OAc$;
MeOOC-C(=CHEt)CH_2-$OOCCH_2$-(*R*)Cgl-Aze-Pab-Z;
Men-$OOCCH_2$-(*R*)Cgl-Aze-Pab-COOPh(4-OMe); and
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2CCl_3$.

32. A compound as claimed in Claim 1 which is
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2CCl_3$;
$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-COO*n*Bu;
n$PrOOCCH_2$-(*R*)Cgl-Aze-Pab-Z;
Cyclooctyl-$OOCCH_2$-(*R*)Cgl-Aze-Pab-Z;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-$COOCH_2OOCCh$;
$MeOOCCH_2$-(*R*)Cgl-Aze-Pab-OH;
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-OH;
n$PrOOCCH_2$-(*R*)Cgl-Aze-Pab-OH;
i$PrOOCCH_2$-(*R*)Cgl-Aze-Pab-OH;

$BnOOCCH_2$-(*R*)Cgl-Aze-Pab-OH; and
$EtOOCCH_2$-(*R*)Cgl-Aze-Pab-OAc.

33. A compound of formula I, as defined in Claim 1, with the additional proviso that R^1 does not represent $-A^1C(O)OR^4$.

34. A compound of formula I, as defined in Claim 1, with the additional proviso that R^4 and R^5 do not independently represent H.

35. A compound of formula I, as defined in Claim 1, with the additional proviso R^6 does not represent C_{1-17} alkyl, when R^2 represents $OC(O)R^6$.

36. A compound of formula I, as defined in Claim 1, wherein R^1 represents $-A^1C(O)OR^4$.

37. A compound of formula I, as defined in Claim 1, wherein R^4 and R^5 independently represent H.

38. A compound of formula I, as defined in Claim 1, wherein R^6 represents C_{1-17} alkyl, when R^2 represents $OC(O)R^6$.

39. A pharmaceutical formulation including a compound of formula I as defined in any one of Claims 1 to 38, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

40. A compound of formula I, as defined in any one of Claims 1 to 38, or a pharmaceutically acceptable salt thereof, for use as a pharmaceutical.

41. A compound of formula I as defined in any one of Claims 1 to 38, or

a pharmaceutically acceptable salt thereof, for use in the treatment of a condition where inhibition of thrombin is required.

42. A compound of formula I as defined in any one of Claims 1 to 38, or a pharmaceutically acceptable salt thereof, for use in the treatment of thrombosis.

43. A compound of formula I as defined in any one of Claims 1 to 38, or a pharmaceutically acceptable salt thereof, for use as an anticoagulant.

44. The use of a compound of formula I as defined in any one of Claims 1 to 38, or a pharmaceutically acceptable salt thereof as active ingredient in the manufacture of a medicament for the treatment of a condition where inhibition of thrombin is required.

45. The use as claimed in Claim 44, wherein the condition is thrombosis.

46. The use of a compound of formula I as defined in any one of Claims 1 to 38, or a pharmaceutically acceptable salt thereof, as active ingredient in the manufacture of an anticoagulant.

47. A method of treatment of a condition where inhibition of thrombin is required which method comprises administration of a therapeutically effective amount of a compound of formula I as defined in any one of Claims 1 to 38, or a pharmaceutically acceptable salt thereof, to a person suffering from, or susceptible to, such a condition.

48. A method as claimed in Claim 47, wherein the condition is thrombosis.

49. A method as claimed in claim 47, wherein the condition is hypercoagulability in blood and tissues.

50. The use of a compound of formula I, as defined in Claim 1 but without the provisos, as a prodrug.

51. A process for the preparation of compounds of formula I which comprises:

(a) for compounds of formula I in which R^2 represents OH, reaction of a corresponding compound of formula I, wherein R^2 represents $OC(O)R^6$ and R^6 is as defined in Claim 1 with an alkoxide base;

(b) for compounds of formula I in which R^2 represents OH, reaction of a corresponding compound of formula I wherein R^2 represents $C(O)OR^7$ and R^7 is as defined in Claim 1 with hydroxylamine, or an acid addition salt thereof;

(c) reaction of a corresponding compound of formula II,

$$\text{H-}(R)\text{Cgl-Aze-Pab-}R^2 \qquad \text{II}$$

wherein R^2 is as defined in Claim 1 with a compound of formula III,

$$R^1O(O)C\text{-}CH_2\text{-}L^1 \qquad \text{III}$$

wherein L^1 represents a leaving group and R^1 is as defined in Claim 1;

(d) for compounds of formula I in which R^1 represents H and R^2 represents OH or $C(O)OR^7$, reaction of a corresponding compound of formula I wherein R^1 represents C_{1-10} alkyl or C_{1-3} alkylphenyl, and R^2 represents OH or $C(O)OR^7$, with a base;

(e) for compounds of formula I wherein R^2 represents $OC(O)R^6$ and R^6 is as defined in Claim 1, reaction of a corresponding compound of formula I wherein R^2 represents OH, with a compound of formula IV,

$$R^6C(O)\text{-}O\text{-}C(O)R^6 \qquad \text{IV}$$

or a compound of formula V,

$$R^6C(O)Hal \qquad \text{V}$$

wherein Hal represents Cl or Br and, in both cases, R^6 is as defined in Claim 1;

(f) for compounds of formula I in which R^1 represents H and R^2 represents $OC(O)R^6$, and R^6 is as defined in Claim 1, reaction of a corresponding compound of formula VI,

$$P^1O(O)C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}R^2 \qquad \text{VI}$$

wherein P^1 represents an acid labile ester protecting group and R^2 represents $OC(O)R^6$, wherein R^6 is as defined in Claim 1, with an acid;

(g) for compounds of formula I in which R^1 represents R^3, R^3 represents C_{1-10} alkyl or C_{1-3} alkylphenyl, and R^2 represents OH or $C(O)OR^7$, and R^7 is as defined in Claim 1 by a trans-esterification of a corresponding compound of formula VII,

$$R^{1a}O(O)C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}R^2 \qquad \text{VII}$$

wherein R^{1a} represents a C_{1-10} alkyl or C_{1-3} alkylphenyl group other than that being formed, or an alternative labile alkyl substituent and R^2 is as defined in Claim 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/01680

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 5/00, A61K 38/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9429336 A1 (ASTRA AKTIEBOLAG), 22 December 1994 (22.12.94) --	1-46,50-51
A	WO 9535309 A1 (BASF AKTIENGESELLSCHAFT), 28 December 1995 (28.12.95) --	1-46,50-51
P,A	WO 9616671 A1 (ASTRA AKTIEBOLAG), 6 June 1996 (06.06.96) --	1-46,50-51
P,A	WO 9614084 A1 (ASTRA AKTIEBOLAG), 17 May 1996 (17.05.96) -- --------	1-46,50-51

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 11 March 1997	Date of mailing of the international search report 12.03.97
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Eva Johansson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE96/01680

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 47-49
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

03/02/97

International application No.
PCT/SE 96/01680

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9429336	22/12/94	AU-A- 6986994	03/01/95
		BR-A- 9406746	19/03/96
		CA-A- 2162900	22/12/94
		CN-A- 1127509	24/07/96
		CZ-A- 9503020	17/04/96
		EP-A- 0701568	20/03/96
		FI-A- 955828	04/12/95
		HR-A- 940311	31/10/96
		HU-D- 9503445	00/00/00
		IL-D- 109634	00/00/00
		JP-T- 8511018	19/11/96
		LT-A- 1947	27/12/94
		LT-B- 3768	25/03/96
		NO-A- 954873	01/02/96
		PL-A- 311819	18/03/96
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WO-A1- 9535309	28/12/95	AU-A- 2787595	15/01/96
		DE-A- 4421052	21/12/95
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		NO-D- 965412	00/00/00
WO-A1- 9616671	06/06/96	AU-A- 4191596	19/06/96
		IL-D- 116153	00/00/00
		SE-D- 9404196	00/00/00
		ZA-A- 9510242	03/06/96
WO-A1- 9614084	17/05/96	AU-A- 3884695	31/05/96
		IL-D- 115879	00/00/00
		SE-C- 504185	02/12/96
		SE-A- 9403831	09/05/96
		ZA-A- 9509377	08/05/96